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Behavior and ecology of Caribbean cleaning gobies *Elacatinus evelynae* in response to reef-condition changes

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RESUMO

Os recifes de coral são um dos ecossistemas mais diversos e complexos do planeta. Os corais são formados por organismos simples que vivem em colónias e em relação simbiótica com zooxantelas, que proporcionam nutrientes em troca de abrigo.

O branqueamento dos corais, que se deve à expulsão das zooxantelas, leva à perda das suas cores vibrantes, sendo substituídas por uma coloração esbranquiçada. Este fenómeno pode conduzir à morte dos corais. Estes eventos, na sua maioria motivados por *stress* térmico, são cada vez mais recorrentes devido às alterações climáticas.

Os parasitas estão presentes e fazem parte da comunidade animal dos recifes, no entanto, evitam o coral vivo, de modo que com a degradação dos recifes e diminuição de coral vivo, os níveis de parasitas podem aumentar. Tal facto leva a um aumento dos níveis de parasitação dos peixes recifais, reduzindo a sua capacidade adaptativa e levando a uma maior vulnerabilidade a infeções.

Um dos mutualismos mais estudado no meio marinho é a relação entre organismos limpadores e peixes maiores que estes, denominados de "clientes". Nesta, os limpadores inspecionam o corpo dos clientes e removem os ectoparasitas neles presentes. Desta forma, o limpador tem acesso a alimento e os clientes veem reduzida a sua carga parasitária, níveis de *stress* e um aumento da sua capacidade imunitária.

Para dar início a uma limpeza, os clientes podem adotar uma pose imóvel, por vezes quase vertical, de forma a indicar ao limpador que querem ser inspecionados; ou o limpador pode iniciar a mesma sem a demonstração de interesse por parte do cliente. Durante a limpeza podem ainda ser removidas escamas e muco, o que é prejudicial para os clientes, uma vez que estas estruturas os protegem de infeções e têm elevados custos de produção, constituindo portanto uma falha de cooperação. Face a este serviço desonesto, os clientes podem efetuar um movimento de "sacudidela" corporal denominado de *jolt*.

Este estudo teve como objetivo observar se a degradação dos recifes de coral implica mudanças de comportamento, dieta ou níveis de *stress* numa espécie de caboz limpador, *Elacatinus evelynae*. Embora esta espécie apresente diferentes níveis de organização social, podendo ser solitários, associarem-se em pares ou em grandes grupos, neste trabalho foram apenas analisados dois tipos de associações: os cabozes solitários e os cabozes que limpam em pares.

Procedeu-se à amostragem de cinco recifes em Curação, uma ilha do sul das Caraíbas. Foram feitos transectos de ponto-intersecção, nos quais foi identificado o tipo de cobertura que aí ocorria (coral vivo, coral morto, coral morto com algas, areia ou outro). Os corais vivos foram posteriormente identificados até ao nível taxonómico mais baixo possível. Foram ainda realizados transectos para analisar a densidade e diversidade da comunidade piscícola. Desta forma, os recifes foram descritos e o seu nível de degradação identificado.

Observaram-se dez estações de limpeza de cabozes limpadores solitários e dez estações de limpeza de cabozes em pares, por recife. O comportamento dos mesmos foi registado considerando: o número de limpezas realizadas, de perseguições pelos cabozes e esperas por parte de clientes que desejavam ser limpos. O tamanho e espécie dos clientes foram também registado. Na ocorrência de uma limpeza, anotou-se a sua duração, quem a iniciava e o número de *jolts* do cliente. Os indivíduos observados foram capturados e transportados para laboratório onde foram eutanasiados e conservados a - 80ºC. Os seus níveis de cortisol foram posteriormente quantificados.

Adicionalmente foram capturados cinco cabozes solitários e cinco pares de cabozes por recife para serem analisados os seus conteúdos estomacais (parasitas e escamas).

As diferenças entre os recifes foram exploradas com recurso a testes de ANOVA, Kruskal-Wallis, ANOSIM, MDS e SIMPER. Foram utilizados ainda testes de Permanova e GLM's para verificar a influência do recife nas diferenças encontradas.

Os cinco recifes amostrados foram separados em três categorias: mais degradado, medianamente degradado e menos degradado. Water Factory foi considerado o recife menos degradado (saudável), uma vez que apresentava a maior cobertura (*ca.* 40%) e diversidade de coral vivo. Carmabi foi considerado o mais degradado uma vez que apresentava a menor cobertura de coral vivo (*ca.* 3 %) e, apesar de também ter a menor cobertura de coral morto, apresentava a maior cobertura de coral morto com algas (*ca.* 70 %). Os restantes recifes (Blue Bay Left, Blue Bay Right e Habitat) foram considerados como estando num estado intermédio de degradação, com cerca de 10 % de coral vivo e 45 % de coral morto coberto por algas.

Nos comportamentos de limpeza só se verificaram duas diferenças: os cabozes solitários tiveram maior número de limpezas no recife saudável; nas estações de cabozes em pares, os clientes esperaram mais frequentemente no recife saudável. Todos os outros comportamentos observados, utilizados como medida da qualidade do serviço e motivações dos cabozes e clientes, não variaram entre recifes em nenhum dos contextos sociais.

Entre os parasitas observados no conteúdo estomacal dos cabozes, foram identificados exemplares de duas famílias: Caligidae e Gnathiidae. Não ocorreram diferenças no consumo de caligídeos entre recifes para os cabozes solitários ou em pares. Já no caso dos gnatiídeos, houve um maior consumo destes por parte dos cabozes solitários num dos recifes de condição intermédia de degradação (Habitat). Nos cabozes em pares o maior consumo foi no recife saudável (Water Factory). No consumo de itens não parasíticos, ou seja, indicador de desonestidade do serviço de limpeza, todos os recifes apresentaram valores semelhantes.

Os cabozes solitários apresentaram valores de *stress* (*i.e.* cortisol) mais elevado no recife degradado (Carmabi). Tal não se verificou nos cabozes em pares, neste caso, os valores foram semelhantes entre todos os recifes.

O recife onde os cabozes habitam é um fator influenciador da sua dieta e níveis *stress*. Isto já não se verifica para os comportamentos observados, para os quais o recife não aparenta ser um fator relevante. No geral, não existiram diferenças entre os cabozes solitários e os cabozes em pares que viviam no mesmo recife.

O maior número de limpezas no recife saudável (Water Factory) não se traduziu num maior consumo de parasitas para os cabozes solitários. Desta forma, é proposta a hipótese de que num recife com elevada parasitação, os cabozes possam efetuar menos limpezas, pois têm acesso a um maior número de alimento por limpeza. Enquanto nos recifes com menor parasitação, os limpadores têm de interagir mais vezes para obter a mesma quantidade de alimento.

A inexistência de diferenças entre as estações de limpeza de cabozes em pares nos vários recifes pode dever-se à preferência dos clientes de serem limpos nas estações de cabozes em pares em detrimento das dos cabozes solitários, uma vez que a limpeza a pares aumenta a honestidade do serviço.

O facto de os cabozes solitários apresentarem diferenças de *stress* entre recifes e os cabozes em pares não, é das primeiras indicações de que os dois grupos reagem de forma diferente à degradação do recife. Adicionalmente, os pares de cabozes apresentam níveis mais elevados de *stress* do que os solitários, exceto no caso do recife mais degradado. Tal pode dever-se a ser mais vantajoso para os cabozes associarem-se a um parceiro nos recifes degradados.

Apesar destes resultados serem promissores, várias questões ainda permanecem por responder. O maior nível de *stress* por parte dos cabozes solitários só se verifica no recife mais degradado, não ocorrendo diferenças entre o recife saudável e os medianamente degradados. Permanece assim a questão de qual o limite da degradação do recife para que esta comece a ter impactos nos cabozes. Estudos futuros devem por isso aumentar o número de recifes a amostrar e englobar mais níveis de degradação de forma a tentar identificar melhor qual é a fronteira para esta influência.

Para a maioria das variáveis amostradas não existiram diferenças entre cabozes solitários e em cabozes pares que habitam dentro do mesmo recife.

Neste estudo, não foi possível confirmar se alguma variável caracterizante do recife tinha mais impacto na influência do mesmo nas diferenças de dieta e *stress*. Foi usado a identidade do recife como um todo, e é por isso relevante que no futuro se explore também esta hipótese.

Este estudo proporciona informação importante para a conservação dos recifes, uma vez que os cabozes limpadores têm um papel ativo nos mesmos, nomeadamente, na manutenção da biodiversidade destes locais.

PALAVRAS-CHAVE: *Elacatinus evelynae*, degradação de recifes de coral, comportamento de limpeza, dieta, *stress*

ABSTRACT

Cleaning interactions are among the most studied mutualisms in the marine environment. They not only have a positive impact on both parts (cleaner and client) but also influence the structure of the reef communities by, among other things, increasing biodiversity.

Sharknose gobies, *Elacatinus evelyane*, and a large number of other cleaning gobies associate with corals, as it is where they maintain their cleaning stations. The increase in coral reef degradation and coral bleaching events affects the parasite proliferation and therefore, goby communities might suffer some kind of impact as well. This study aimed to understand how reef degradation affects these cleaning gobies' behavior, diet and stress levels.

Five reefs were sampled in Curaçao, South Caribbean. Three different reef health conditions were established - degraded, fair and healthy - by analyzing the fish community (density and diversity), coral diversity and the substratum cover (live and dead coral, sand, and algae). Behavior, diet and stress levels were sampled for both single and paired gobies.

Although some differences in behavior for both single and paired gobies were found between reefs, in healthier reefs single gobies had more cleaning interactions than the degraded one, and paired gobies had more client waits than the fair reefs. However, it seems these were not due to the differences in reef health conditions.

The reef condition was in fact influencing the gobies diet and stress levels. For the diet, single gobies in one of the fair reefs had more intake of parasites than in all the other reefs, and for the paired gobies the higher intake was for the healthier reef. As for stress levels, single gobies were indeed more stressed in the degraded reef than in the fair and healthy ones, but these differences were not observed in paired gobies, as stress levels were similar in all reefs. This indicates that perhaps single and paired gobies react differently to different reef degradation stages, and that it might be more advantageous for gobies in degraded reefs to clean with a partner.

KEY-WORDS: *Elacatinus evelynae*, coral reef degradation, cleaning behavior, diet, stress

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ABBREVIATIONS

BBL	Blue Bay Left
BBR	Blue Bay Right
C	Carmabi
H	Habitat
WF	Water Factory
CS	Cleaning stations
DC	Dead coral

1. INTRODUCTION

1.1. Coral Reefs

Coral reefs are among the most diverse and complex ecosystems worldwide. They support 25% of all marine life, even though they only represent 0.2% of the ocean floor cover (Spalding et al. 2001).

Corals are small and simple organisms and can be found in all oceans and at all depths. A large number of species live in large colonies and have the ability to build a shared skeleton. These skeletons can be made of calcium carbonate, making them hermatypic or reef building corals. With only a few millimeters of growth per year, hermatypic corals are frail organisms with a slow growth rate even in ideal conditions, and can be destroyed in only a few hours (*e.g.* hurricanes) (Spalding et al. 2001).

The majority of coral species have a symbiotic relationship with zooxanthellae, providing them shelter while receiving nutrients from their photosynthesis. It is considered that most corals are dependent of these organisms, which in the case of bleaching events is worrisome. Bleaching occurs when the zooxanthellae are expelled or when they lose their chlorophyll. This leads to the loss of corals vibrant colors becoming white or pastel colored (Spalding et al. 2001).

Even though several factors can cause bleaching, the great majority are temperature-related stressors, that are increasing with climate change (Spalding et al. 2001). Coral recovery from bleaching events can occur, although sometimes it is not possible and they perish, turning into rubble and/or becoming covered with sponge and algae, which leads to major reef structure changes (Gardner et al. 2003; Artim and Sikkel 2013). This replacement of live coral for algae is usually known as coral-algae phase shift (Hughes 1994; Bruno et al. 2009) and has an effect on fish assemblages (Done 1992), on fish and invertebrate diversity (Idjadi and Edmunds 2006), and on ectoparasite density (Artim and Sikkel 2013). In addition to this, turf algae directly affects neighboring corals by weakening them, reducing coral fitness and outgrowing them (Birrel et al. 2005; Quan-Young and Espinoza-Avalos 2006; Titlyanov et al. 2007; Barott et al. 2009; Vermeij et al. 2010). It also prevents the settlement of new coral planulae, which leads to a progressive reef degradation that corals cannot overcome (Vermeji and Sandin 2008; Vermeij et al. 2010).

Additionally, reefs are also affected by a plenitude of other conditions such as pollution, coral disease, over-harvesting, storms, nutrient excess, coastal development and sediment pollution (Pandolfi et al. 2003; Bellwood et al. 2004; Hughes et al. 2007; Wilkinson 2008).

Coral degradation is a worldwide crisis, with 19% of the original area of coral reefs already lost (Wilkinson 2008) and with 75% of the world's reefs threatened (Burke et al. 2011). If no measures are taken this percentage will increase to more than 90% by 2030 and to virtually 100% by 2050 (Burke et al. 2011).

1.2. The Caribbean – a region in serious risk?

The Caribbean represents 7% of the world total reef area and is one of the sites with most reports of reef degradation. It is reported that more than two thirds of Caribbean reefs are threatened, with a considerable part in high and very high risk categories (McClanahan et al. 1999; Spalding et al. 2001; Burke and Maidens 2004; Bruno et al. 2009). In only 30 years, the average hard coral cover on the Caribbean reefs went down from 50% to 10% (Gardner et al. 2003).

Caribbean coral reefs appear to have a lower resilience in relation to other reefs, due to a higher macroalgae productivity (Roff and Mumby 2012). This makes the reefs more prone to suffer algae blooms. In addition, Caribbean functional groups are less diverse and thus if a species disappears, there is a lower capacity to replace its role in the ecosystem (Bellwood et al. 2004). All of this makes Caribbean reefs more frail, vulnerable and their rehabilitation a harder process (Gardner et al. 2003; Bellwood et al. 2004; Roff and Mumby 2012).

The chosen study site, the island of Curaçao, is located in the southern Caribbean, north to the coast of Venezuela. It is surrounded by fringing reefs, where more than 70% of all Caribbean species can be found, making the island one of the most diverse areas in the Caribbean (Vermeij 2012). In addition, growing reefs still exist on the island, making this archipelago even more unique. Thus, the coral reefs of Curaçao represent one of the best reef systems left in the Caribbean at present. Yet they also face the same worldwide pressures: coastal development, tourism overuse, pollution and the rise in sea temperature due to climate change (Vermeij 2012). In the past, reef building corals covered approximately 40% of the reefs in Curaçao (Duyl 1985). By 2010 that percentage had decreased by half (Vermeij 2012). If this decline rate continues, even though growing corals still exist, some estimates predict that coral reefs in Curaçao will decline and disappear by 2060 (Vermeij 2012).

One of the largest threats to coral reefs is the rise in sea temperature, which leads to bleaching events (Wilkinson 2008). In 2010 occurred the worst coral bleaching event ever reported in Curaçao, with 12% of reef building corals being affected (in some locations it went up to 30%). It surpassed the 2005 event that was a record setting for the Tropical Atlantic and Caribbean (Vermeij 2012). This suggests that coral bleaching will start occurring regularly, giving more importance to studies that investigate the impacts that these changes can have (Vermeij 2012).

1.3. The importance of positive mutualistic interactions to coral reef ecosystems

Mutualisms are, by definition, interspecific relationships that result in benefits for all parties involved (Côté 2000). Positive mutualistic interactions are considered to be of key importance for reef fish communities as these make the environment, directly or indirectly, more favorable for associated species, which in turn will facilitate the establishment of other species (Grutter and Irving 2007).

At larger regional scales, positive interactions enhance diversity via an increase in habitat diversity (Stachowicz 2001). At a more individual scale, these interactions seem to contribute to an increase of body condition and decreased baseline and acute stress levels.

Cleaning mutualisms can take two forms: an incidental cleaning, which does not require any adaptation on either of the participants, or a cleaning that involves specific behavior and morphology (Côté 2000). An example of the first case occurs between herbivore fishes and sea turtles. In this association the fish eat algae lodged on the body surface of the turtle, just as if they would from the substratum (Losey et al. 1994). The second case involves specialized organisms, known as cleaners, that receive the visit of other often larger organisms (known as clients), to inspect their body in search for ectoparasites, mucus and dead or diseased tissues (Feder 1966; Côté 2000).

During mutualistic cleaning interactions, the cleaners feed on ectoparasites, such as gnathiids and caligid copepods (Arnal and Côté 2000). If not removed, these can lead to host disease, behavior and immunological changes, host fitness reduction, and can even impact host population dynamics (Arnal et al. 2000; Barber et al. 2000; Buchmann and Lindenstrøm 2002; Finley and Forrester 2003; Hudson et al. 2006). Through this, the cleaner gets access to a preferred meal and the clients get their parasite load reduced, preventing or reducing the effects referred above which have a major impact in fish health (Grutter 1999; Arnal and Côté 2000; Arnal et al. 2000; Bshary et al. 2007).

These cleaning interactions promote local reef fish diversity and density, and impacts the structure of reef fish communities (Bshary 2003; Grutter et al. 2003; Bshary et al. 2007).

The sequence of a cleaning interaction may start either with the client posing for the cleaner or with the cleaner first approaching the client (Côté 2000; Soares et al. 2007). Client poses are usually very conspicuous, since clients adopt an immobile posture. All fins are erect and the body almost vertical, remaining motionless throughout all the interaction (Côté et al. 1998). Species have specific poses, although some show variability in their posing behavior (Côté et al. 1998). Cleaners then start inspecting the client’s body surface, inside the buccopharyngeal cavity and the gills chamber, removing ectoparasites, dead or infected tissue and debris (Grutter and Hendrikz 1999; Grutter 2002).

Cleaners can remove more than ectoparasites. Sometimes they also remove healthy tissue, mucus and/or scales (Feder 1966; White et al. 2007; Soares et al. 2008a; Campos and Sá-Oliveira 2011). Such behavior is labelled as “cheating”, since mucus protects fish against infections, and due to a high protein content, it is expensive to produce not being beneficial for the client (Ebran et al. 1999; Arnal et al. 2001).

Clients keep track of cleaners’ behavior, if the latter provides an honest (remove parasites) or a dishonest (cheat) service. From the client’s perspective, getting immediately inspected and receiving a good service are factors that can evaluate the quality of a cleaning interaction (Bshary and Noë 2003). Cleaners cheating behavior may be measured by jolt count, which are whole-body shudders that are apparently painful reactions to a cleaner fish bite and have been associated to dishonest bites by cleaners (Soares et al. 2008a).

Nevertheless, clients benefit from interacting with cleaners, as it reduces parasite loads, decreases stress levels and the need to have a high immune function. Lower loads and exposure to parasites lead to a reduction in the energy allocated to the immune system, that can be used for other processes such as growing (Bshary et al. 2007; Ros et al. 2011a). Stress reduction may even be a key motivation for clients to seek cleaners (Ros et al. 2011b). Another interesting effect arises from the basic access to physical contact, which also contributes to the reduction of baseline stress levels in clients (Soares et al. 2011).

1.4. Cleaning gobies

Cleaning gobies (*Elacatinus* spp.) are the most ubiquitous cleaners in the Caribbean region (Soares et al. 2008a). This study focused on sharknose gobies, *Elacatinus evelynae* (Böhlke and Robins, 1968), which are relatively small (1.2-3.5 cm total length) and have a prominent lateral stripe (blue and yellow) extending from the snout to the tail (Soares et al. 2009).

Although best known for their cleaning behaviors they are sometimes described as facultative cleaners by some authors, due to their alternative habitat related strategies, which may vary between coral heads or basket sponges (Côté 2000; White et al. 2007). When found on coral heads they are cleaners and that is where they maintain their territories (known as cleaning stations) (White et al. 2007). If found on basket sponges, they feed mainly on nonparasitic copepods and spend little time cleaning (White et al. 2007). In this study, only cleaners found on coral heads were considered.

Sharknose gobies can be found alone, in pairs or in groups in their territories and their behavior is known to vary in accordance to their social status (Soares et al. 2008a, 2009).

As in other cleaner species, both scales and mucus have been found in *E. evelynae* stomach, demonstrating the practice of a dishonest service. However, contrarily to *Labroides dimidiatus*, the Bluestreak cleaner wrasse, one of the most abundant and well known cleaner in the Pacific, gobies appear to prefer eating ectoparasites (Soares et al. 2010). So it is suggested that gobies start their cleaning interaction just searching for this food item but once it is reduced they start ingesting mucus and scales (Soares et al. 2010).

In order to control dishonest cleaners, clients may either change the cleaning station they visit or respond aggressively by chasing the cleaner (Soares et al. 2008c). On the other hand, some cleaners also manipulate clients into staying after cheating occurs, by providing tactile stimulation with their pelvic fins to the clients’ dorsal region (Soares et al. 2008c). Cleaning gobies however do not have either (Soares et al. 2008c). They do not perform tactile stimulation because they are ultimately honest. This leads to a minimum amount of conflict and to an overall similar quality of service, therefore clients do not need to punish them. Moreover, in the cleaning goby system, clients’ jolts act as a reliable signal that the parasite load is low and that it is time to stop the interaction (Soares et al. 2008c, 2010).

1.5. Gobies in stress: how are cleaning mutualisms coping?

Stress can be described as "the nonspecific response of the body to any demand made upon it" (Selye 1973, p. 692). It can also be described as a state of threatened homeostasis by a real or perceived stressor that is re-established by a complex suite of adaptive responses (Chrousos 1998; Barton 2002).

Stressors can vary from predation risk and other antagonistic fish interactions to environmental variables. The degree of reaction to a stressor is variable between species. Some have a high reaction and others an almost unnoticeable one (Iwama 1998; Barton 2002; Soares et al. 2012). However if the stressor is prolonged and severe, it can have an impact on fish health and well-being, from a molecular level to its social organization (Barton and Iwama 1991; Barton 2002).

Fish response to stressors is divided in primary, secondary and tertiary responses (Iwama 1998; Barton 2002). The first include endocrine changes such as the release of catecholamines and corticosteroids (Wendelaar Bonga 1997; Reid et al. 1998; Barton 2002). The second involves metabolic, cellular, hematological and immune function changes and osmoregulatory disturbance (Iwama 1998; Barton 2002). And tertiary responses are related to changes in whole-animal performance and modified behavioral patterns (Iwama 1998; Barton 2002).

Corticosteroids are able to cross blood-brain barrier and access the receptors in the brain. This adds to their importance in stress responses, because, in order to affect behavior, the mediation of stress also has to affect the brain (Nelson 2005; Soares et al. 2012).

Cortisol is the main corticosteroid in teleosts and, contrarily to the catecholamines that are immediately released after a fish is exposed to a stressor, cortisol release is delayed and its effects are more prolonged (Gamperl et al. 1994; Barton 2002; Martinez-Porchas et al. 2009). This allows to sample and measure fish baseline levels of cortisol, and therefore it is commonly used as an indicator of stress in fish (Barton and Iwama 1991; Wendelaar Bonga 1997).

Various studies investigated the relation between the access to cleaning interactions and the clients' stress levels (Bshary et al. 2007; Ros et al. 2011a). However, few attempted to comprehend cleaners' stress levels, their variations, and which variables can sway such levels.

Munday (2004) proposed that habitat specialists would be the first species to be lost from coral reefs if degradation occurs. The fact that some cleaning gobies are coral dwellers could mean they are more predisposed to be affected by the degradation in coral reefs. Thus, considering the real pressures of organisms inhabiting coral reefs, particularly those considered to have a key relevance to the community, it is important to know how these are responding to ecosystem shifts, the lowering of fish diversity and changes in food (parasite) availability.

1.6. Study objectives

The importance of cleaners, their behavioral abilities and their scope of influence to the remaining fish communities is clearly linked to a necessity to find out more about the underlying mechanisms of these elaborate interactions and how exposed these may be to the present dramatic changes suffered by reef ecosystems. Thus, cleaners are under a significant amount of pressure. Also, the relevant changes in their fish clientele and parasite levels may also translate into cleaning goby physiology changes.

Thus, taking this into consideration, this study aims to understand if gobies living in different reefs exhibit:

(1) different behaviors;
(2) different diet and food consumption;
(3) different stress levels;
(4) if these differences are influenced by the quality of the reef they inhabit.

2. MATERIALS AND METHODS

2.1. Study sites and species

This study took place in the southwestern coast of Curaçao, Netherlands Antilles, between June and August 2014. Five reef sites (Figure 2.1), Habitat, Blue Bay Right, Blue Bay Left, Carmabi and Water Factory, were chosen based on visual differences in reef health status and the report 'The current state of Curaçao's Coral Reefs' by Mark Vermeij (2012), with the attempt to maximize the differences in fish abundance, diversity and benthic characteristics amongst reefs.

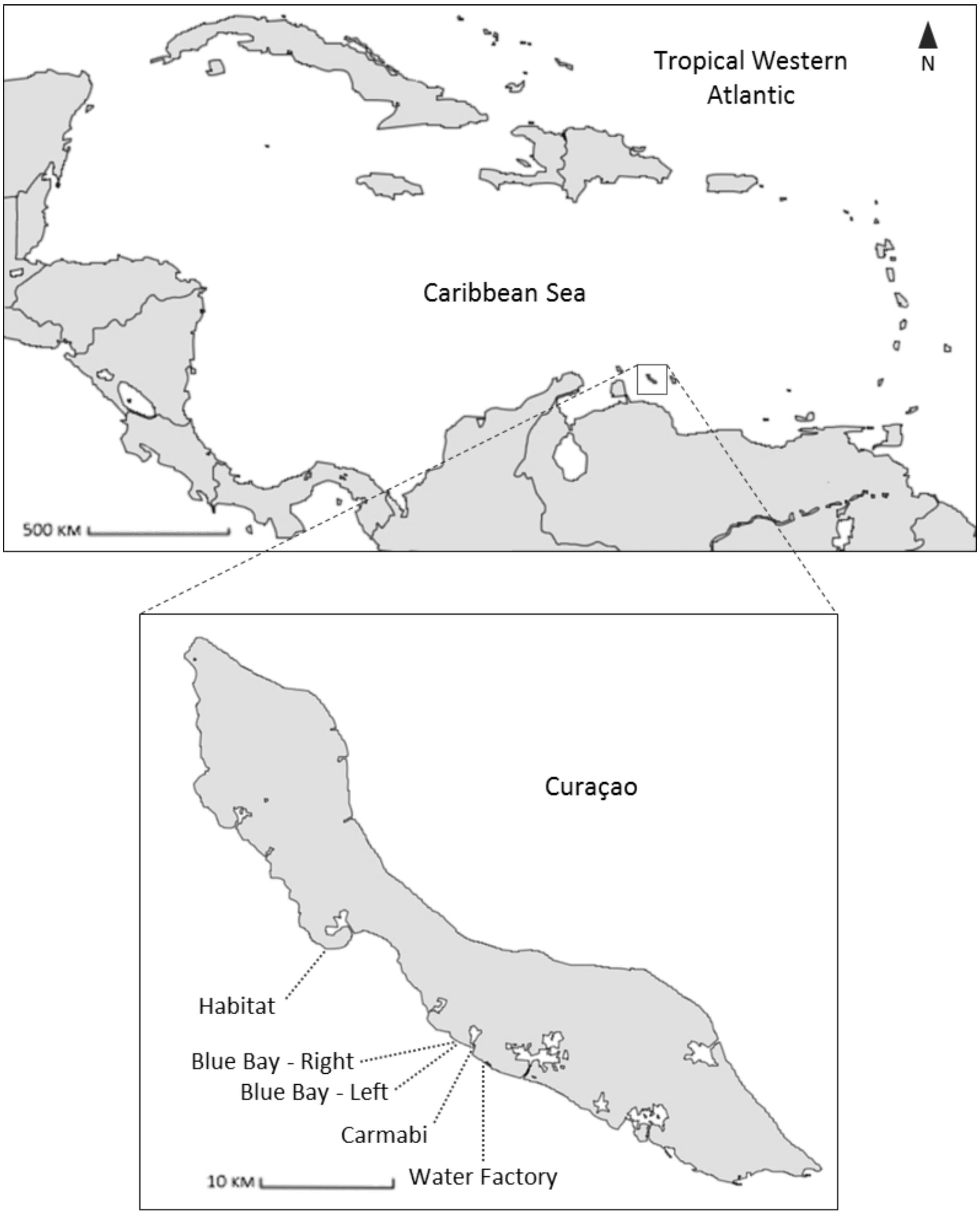

Figure 2.1- The Island of Curaçao, and the location of the studied reefs (Santos 2016).

The reefs sampled were located on the south west side of the island, which is characterized for having a gradually sloping terrace in front, dropping-off at 7 m to 12 m with a slope that varies from 45° to vertical (Bak 1975). Coral cover and diversity are at the highest near the drop-off, reaching 70% (Vermeij 2012). The studied reefs were distributed along a 21 km stretch of coastline, with distances between reefs that ranged between 300 m to 12 500 m.

The target species in this study was the sharknose cleaning goby, *Elacatinus evelynae* (Figure 2.2). It is the main cleaning species in the Caribbean, with a high abundance and is easily identifiable (White et al. 2007; Soares et al. 2008c, 2012).

Figure 2.2 – Sharknose cleaning goby, *Elacatinus evelynae*.

2.2. Coral reef diversity and abundance

Point intersect transects were used to access the quantity and diversity of corals in all the selected five reefs. Ten 10 m long transects were carried out per reef by one diver. In order to categorize the dominant substrate (dead coral, dead coral with algae, live coral, sand and other) annotations were taken every 10 cm, totaling 101 per transect. All living corals were photographed for posterior identification; they were identified to the lowest taxonomical level possible.

2.3. Reef fish diversity and density

In order to analyze reef fish diversity and density, six transects were performed in each reef. Each transect had a total length of 50 m, a width of 4 m and was conducted by two divers. The transect was marked longitudinally with a measuring tape placed in the center of the 4 m width. An assessment dive was performed in which all species observed were photographed and/or described in a plastic board so they could be later identified and a species list compiled. This list was divided in two, so each diver was responsible to count the individuals of a different set of species. The 50 m distance was swam twice.

The first time, the species on the list were noted and the number of individuals per species was counted. The second time around, all the cleaning stations and *E.evelynae* were counted, in this case each diver would get one side of the sampled area (right or left side of the measuring tape). If new species to the list were observed along a transect, a photo or description was taken to allow a posterior identification and addition to the list.

2.4. Cleaning gobies behavioral observation

For each reef a total of 20 cleaning stations were observed, 10 occupied by one adult cleaning goby (single) and 10 operated by two adult cleaning gobies (a pair). The observations were done by three different divers. In order to reduce the error between different observers, preliminary observations were done to ensure that the criteria utilized by all divers was standardized.

The cleaning stations (and thus the cleaners) were chosen haphazardly, between depths of 1 m and 15 m. Each cleaning station was observed once for a period of 20 min, between 10 am and 4 pm, which coincided with the period of cleaning activity in this species (Johnson and Ruben 1988). Observations were made from a distance of 2-3 m. A total of 2000 min of observations were logged by the divers during this study.

All cleaner fish were observed per cleaning station (one or two in the case of a pair). During each observation the species and size (total length estimated to the nearest cm) of each visiting client was registered and, three types of behavior were recorded: waiting to be inspected (when a client is willing to be cleaned and waits to be attended, but it is not), cleaning interactions (when a client is inspected) and cleaner chases (whenever a cleaner pursues a client, trying to start a cleaning interaction, but does not) (Soares et al. 2008a, 2009). Whenever an interaction occurred, its duration was registered as well as which party initiated each interaction: whether the client posed before receiving an inspection (client-initiated) or if the cleaner began the inspection prior to client posing behavior (cleaner-initiated). Finally, the number of jolts performed by the client and if the interaction was terminated with a jolt were also recorded (Soares et al. 2008a, 2009).

2.5. Cleaning goby diet analysis

On each of the focused reefs, 5 singles and 5 pairs of cleaning gobies (in a total of 15 individuals) were randomly sampled. In the lab, individuals were transferred to Eppendorf vials with 90% alcohol for further preservation.

Cleaning gobies were then dissected and intestines and stomach were analyzed (White et al. 2007). The items found were counted and categorized as ectoparasites (identified as the parasitic copepod -Caligidae and the parasitic isopod -Gnathiidae), scales, and non-parasitic items (mainly free living copepods). Due to the low numbers of food items, they were counted individually.

2.6. Tissue Cortisol extraction and determination

To evaluate tissue cortisol levels as a stress response indicator, after each behavioral observation (see point 4 above), cleaning gobies were immediately captured with a hand net, placed in a zip plastic bag and were taken to the lab where they were submerged in a mixture of clove oil, ethanol and sea water (1/4 clove oil, 1/4 ethanol, 2/4 sea water), used for anesthesia purposes. The individuals were then measured and rapidly killed by severing the spinal medulla. The brain was dissected and stored in

Eppendorf vials at -80°C and the body was separately stored, also at -80°C. The whole body of the collected gobies (without the brain) was used to perform cortisol analysis. The gobies' bodies were weighed and then homogenized with 500µL of PBS, after which ethanol was added. The amount of ethanol added varied with the goby body weight. To select the correct amount of ethanol the following equation was used:

$$(body\ weight\ (\mathrm{g}) + 500) \times 3 = ethanol\ volume\ (\mu\mathrm{L}) \tag{2.1}$$

The sample was then homogenized with the vortex and left overnight at 4°C. Afterwards the sample was concentrated through ethanol evaporation forced by an injection of nitrogen gas. Finally, a cortisol radioimmunoassay (RIA) was conducted.

2.7. Statistical analysis

From the collected data, some calculations were made in order to perform the statistical analysis. These can be categorized in goby behavior and reef descriptors.

a) Reef health status descriptors:

$$\begin{matrix} percentage\ of\ cover \\ of\ benthic\ category \end{matrix} = \frac{No.of\ ocurrences\ \times 100}{101} \tag{2.2}$$

b) Goby behavior calculations:

$$\begin{matrix} client\ species\ frequency \\ at\ cleaning\ stations \end{matrix} = \frac{No.of\ visits\ by\ a\ species}{average\ species\ density\ \ (transect)} \tag{2.3}$$

$$\begin{matrix} likelihood\ of\ client \\ posing\ success \end{matrix} = \frac{No.cleaning\ events\ that\ started\ with\ a\ pose}{total\ \ no.\ of\ \ poses} \tag{2.4}$$

$$\begin{matrix} proportion\ of\ cleaning\ events \\ started\ by\ the\ cleaner \end{matrix} = \frac{No.cleaning\ events\ started\ by\ a\ cleaner}{total\ \ no.\ of\ cleaning\ events} \tag{2.5}$$

$$\begin{matrix} likelihood\ of\ chase \\ by\ goby\ sucess \end{matrix} = \frac{No.cleaning\ events\ that\ started\ with\ a\ chase}{total\ no.of chases} \tag{2.6}$$

$$percentage\ of\ cheating\ = \frac{No.of\ scales\ ingested}{No.of\ scales\ and\ parasites\ ingested} \times 100 \tag{2.7}$$

Using PRIMER 6, Primer-E, Ltd an analysis of similarity (ANOSIM), a multidimensional scaling (MDS) plot and a similarity percentage analysis (SIMPER) were performed to differentiate the reefs. In order to create a Bray-Curtis resemblance matrix, needed for the ANOSIM, the abundance matrix for the benthic structure data was square-rooted and the fish species data fourth rooted. Furthermore a permutational multivariate ANOVA (PERMANOVA) followed by pairwise tests, was used to analyze differences in goby stomach content, for single and paired gobies separately. To do so, a resemblance matrix was built using Bray-Curtis similarity.

The remaining analyses were performed using IBM SPSS Statistics 22. The assumptions for normality and homoscedasticity were tested for all data. If assumptions were met, one-way ANOVA were performed. If they were not met, even after transformation, Kruskal-Wallis tests were used. Tukey post-hoc tests and Dunn´s post-hoc tests, followed respectively. Finally generalized linear models (GLMs) were performed, of which the models with the lowest AIC were chosen. For all GLMs normal probability distribution and the link function identity was used, and aside from the logarithmization of the interaction duration, no transformations were needed.

3. Results

3.1. Reef description

The sampled reefs had different cover percentage of the sampled benthic categories (Figure 3.1a). Water Factory had a higher percentage of live coral cover than all the other reefs (one-way ANOVA, $F_4 = 34.895$ $p < 0.001$, Tukey test $p < 0.001$). Habitat and Blue Bay Left had as well a higher percentage of live coral cover than Carmabi (Tukey test, H-C $p = 0.021$; Tukey test BBL-C $p = 0.005$). Dead Coral cover also varied across reefs (Kruskal-Wallis, $H_4 = 24.767$, $p < 0.001$), with Carmabi having lower cover than Blue Bay Left (Dunn's test, $p = 0.003$), Habitat (Dunn's test, $p = 0.005$) and Water Factory (Dunn's test, $p < 0.001$). Carmabi has more cover of dead coral with algae than Water Factory (Kruskal- Wallis, $H_4 = 20.206$, $p < 0.001$, Dunn's test $p < 0.001$). For the sand and other categories, cover percentage was different across reefs but the pairwise tests were not able to identify the reefs responsible for this (% Cover Sand: Kruskal-Wallis, $H_4 = 9.906$, $p = 0.042$, Dunn's test $p > 0.05$; % Cover Other: Kruskal-Wallis, $H_4 = 9.867$, $p = 0.043$, Dunn's test $p > 0.05$).

In total, 24 species of corals were identified and 4 corals were only identified to the genus. Water Factory had a higher diversity of coral species than all the other reefs (one-way ANOVA, $F_4 = 14.820$, $p < 0.001$, Tukey tests $p < 0.001$) (Figure 3.1b)

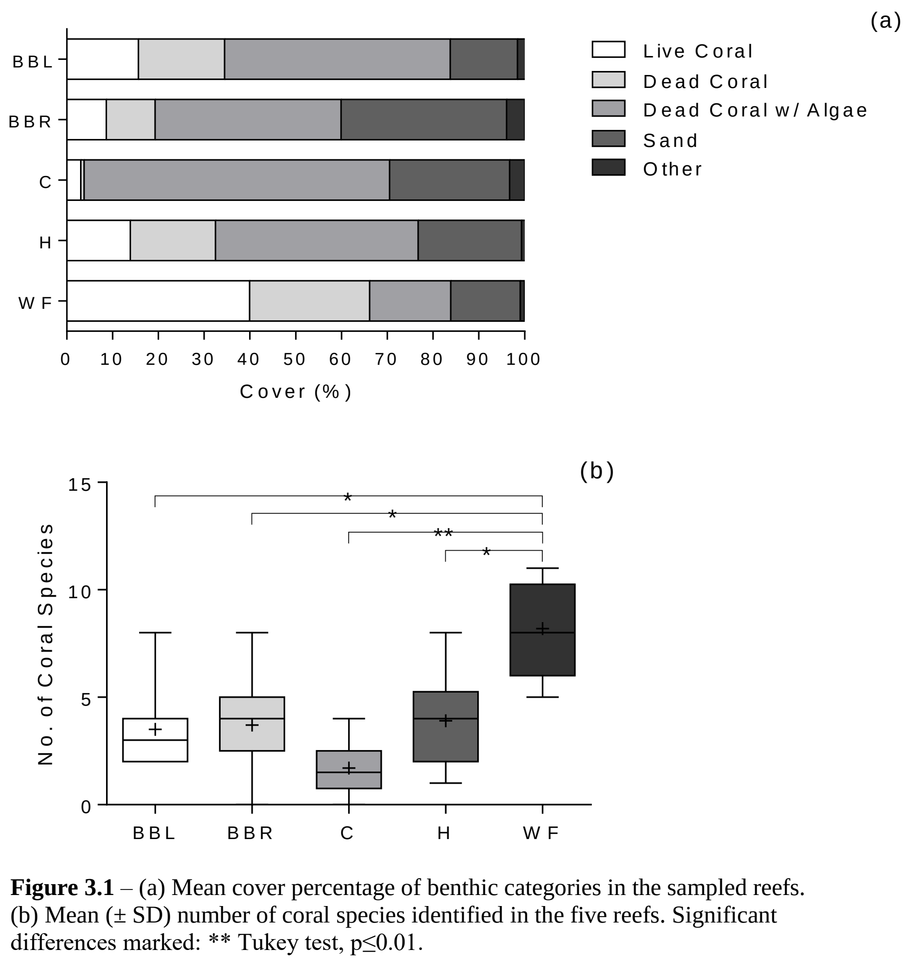

Figure 3.1 – (a) Mean cover percentage of benthic categories in the sampled reefs. (b) Mean (± SD) number of coral species identified in the five reefs. Significant differences marked: ** Tukey test, p≤0.01.
Sample size: $n_{BBL} = n_{BBR} = n_C = n_H = n_{WF} = 10$

Taking into consideration all benthic categories sampled and the species identified in the reefs, significant differences between reefs can be found (one-way ANOSIM $R = 0.391$, $p = 0.001$). To better visualize how, a MDS was performed, where each point represents a transect in the reef (Figure 3.2). A clear separation between Carmabi and Water Factory can be seen. Carmabi shows to be the more homogenous reef since all transects are closer to one another, on the contrary, Habitat is the more heterogeneous reef. Blue Bay Right and Left, although geographically close, do not have a lot of overlapping, and appear to be between Carmabi and Water Factory. On the other hand, Habitat has a lot of overlapping with Blue Bay Left and Right and some with Carmabi. This goes in accordance with the ANOSIM pairwise tests, apart from the similarities between Habitat and Blue Bay Left ($p = 0.139$) and Right ($p = 0.073$), the remaining reefs were different from one another.

The SIMPER analysis allows to identify the dissimilarity percentage between the reefs when considering the sampled benthic categories, this showed that Water Factory and Carmabi are the most dissimilar reefs, with 64.25%. of dissimilarity.

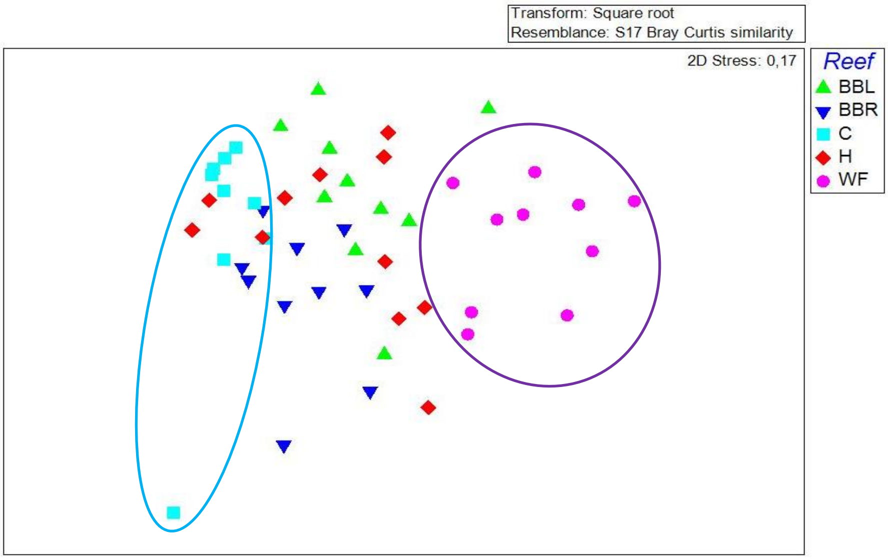

Figure 3.2 - Multidimensional scaling plot of benthic community across reefs. Each point corresponds to a single transect done in Habitat (H), Blue Bay Right (BBR), Blue Bay Left (BBL), Carmabi (C), and Water Factory (WF). Sample size: 10 transects per reef.

For the total number of cleaning stations per square meter, significant differences were found between reefs (one-way ANOVA, $F_4=12.696$, $p<0.001$) (Figure 3.3). Carmabi had less cleaning stations than Blue Bay Left, Blue Bay Right and Water Factory (Tukey test, $p < 0.05$) and Habitat had less cleaning stations than Blue Bay Right (Tukey test, $p = 0.001$) and Water Factory (Tukey test, $p < 0.011$).

Results are almost identical regarding the comparisons for the number of cleaning stations of single and paired gobies between reefs. For single gobies cleaning stations (Figure 3.3), Carmabi remained with significantly less cleaning station than Water Factory (one-way ANOVA, $F4=10.455$, $p<0.001$; Tukey test, $p<0.001$), Blue Bay Left (Tukey test, $p= 0.001$) and Blue Bay Right (Tukey test, $p<0.001$). However in this case Habitat is solely statistically different from Blue Bay Right, with again less cleaning stations than the latter (Tukey test, $p=0.006$). Regarding the paired gobies cleaning stations (Figure 3.3), Carmabi again has less cleaning station than all the other reefs except Habitat (one-way ANOVA, $F4=7.892$, $p<0.001$; Tukey test, $p<0.05$) while Habitat has less cleaning stations than Water Factory (Tukey test, $p=0.032$) and Blue Bay Left (Tukey test, $p=0.038$).

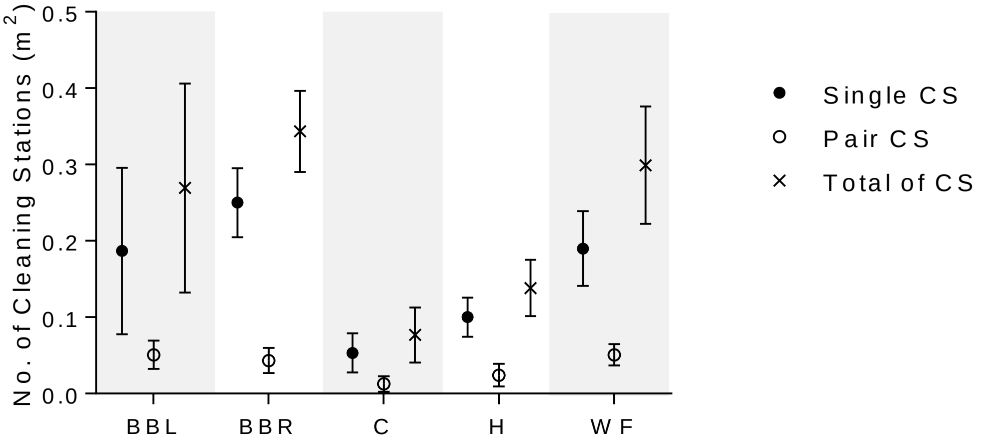

Figure 3.3 – Mean (± SD) number of cleaning stations of: (●) single goby, (○) paired gobies , (✕) total number of cleaning stations. Sample size for all variables: n_{BBL}= n_{BBR}= n_C=n_H = n_{WF}=6.

Overall, the number of *Elacatinus evelynae* observed differed across reefs (one-way ANOVA, F_4=10.766, p<0.001) (Figure 3.4), which was in conformity with the results presented above. Again Carmabi had less *E. evelynae* than Water Factory (Tukey test, p=0.001), Blue Bay Left (Tukey test, p=0.025) and Blue Bay Right (Tukey test, p<0.001),and so does Habitat when compared to Water Factory (Tukey test, p=0.003) and Blue Bay Right (Tukey test, p=0.002)-

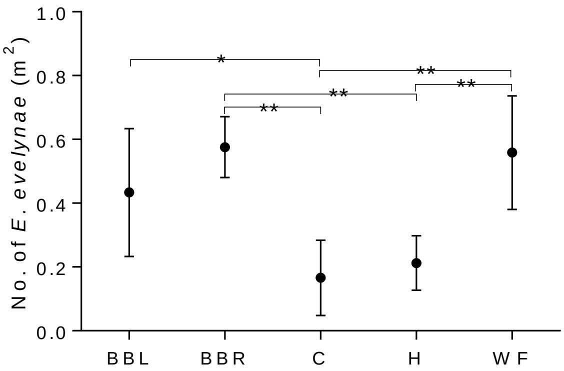

Figure 3.4 – Mean (± SD) *E.evelynae* density per m^2 in the five sampled reefs. Significant differences are marked with: * Tukey test, p<0.05; ** Tukey test, p<0.01. Sample size: n_{BBL}= n_{BBR}= n_C=n_H = n_{WF}=6.

Finally, concerning the reef fish communities, 87 fish species were identified across the 5 reefs. Both density and diversity varies across some reefs (fish density: one-way ANOVA, F_4=5.055, p=0.004; fish diversity: one-way ANOVA, F_4=5.091, p=0.004, Tukey test, p<0.05) (Figure 3.5a). Carmabi had a lower density than Habitat (Tukey test, p=0.016) and Blue Bay Right (Tukey test, p=0.019). Additionally, it was also less diverse than Blue Bay Left (p = 0.044) and Blue Bay Right (p = 0.003). Water Factory on the other hand had a lower fish diversity than Blue Bay Right (p = 0.049) (Figure 3.5b).

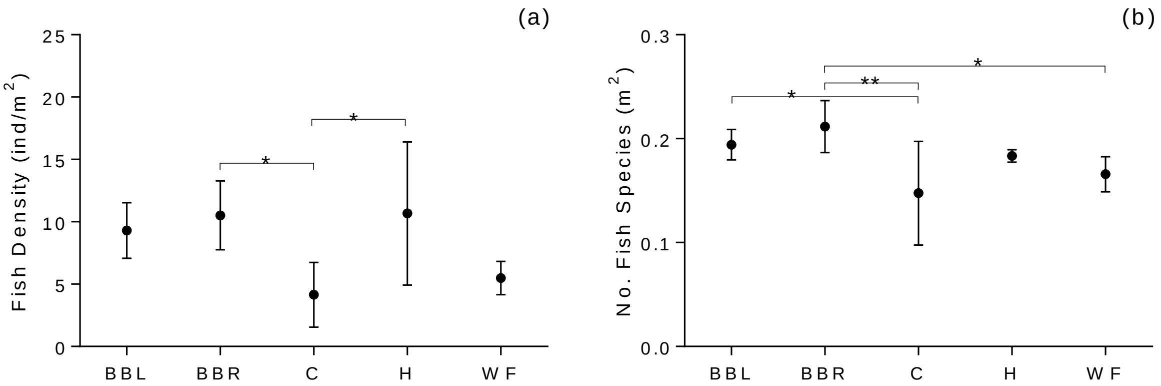

Figure 3.5 – Mean (±SD) fish density (a) and fish diversity (b) in the five sampled reefs. Significant differences are marked with: : * Tukey test, p<0.05; ** Tukey test, p<0.01. Sample size: $n_{BBL}= n_{BBR}= n_C=n_H = n_{WF}=6$.

When taking both fish density and diversity into consideration, the fish community was significantly different between all reefs (one-way ANOSIM, R=0.5, p=0.001, pairwise tests p<0.002). In Figure 3.6, one is able to confirm that Carmabi was both separated and showing the highest transect heterogeneity, in opposition to Water Factory which was also separated but more consistent between transects. Blue Bay Left, Blue Bay Right and Habitat look the most similar reefs, showing all the points closer and mostly overlapping each other. Once again the SIMPER analysis identified Carmabi and Water Factory as the reefs with the highest dissimilarity (45.82%).

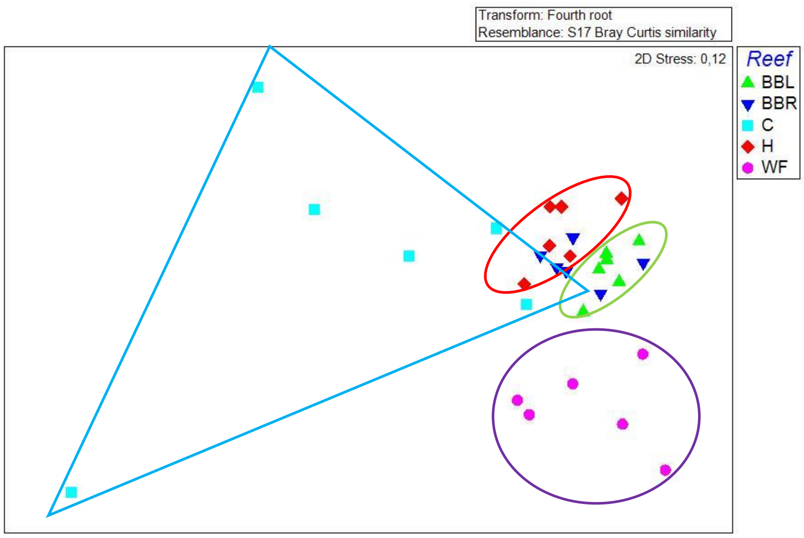

Figure 3.6 - Multidimensional scaling plot of the fish community across the reefs. Each point corresponds to a single transect done in each reef. Sample size: 6 transects per reef.

The frequency of different client species at the single gobies cleaning stations was not significantly different (one-way ANOSIM, R = 0,028, p = 0.186). However for the paired gobies cleaning stations some reefs showed significant differences (one-way ANOSIM, R = 0.159 p = 0.001)[1] (Table 3.1), with Water Factory proving to be different to all the reefs ($p < 0.05$), and Carmabi to Blue Bay Left (p = 0.024) and Blue Bay Right (p =0.005).

Table 3.1– Total number of client species observed at single and paired cleaning stations

Reef	Single CS	Pairs CS
Blue Bay Left	9	9
Blue Bay Right	9	11
Carmabi	9	9
Habitat	14	7
Water Factory	5	6

3.2. Cleaning goby behavior

3.2.1. Single cleaning gobies

Carmabi was the reef where single gobies had fewer cleaning interactions and Water Factory the reef where cleaning gobies interacted the most, (Kruskal-Wallis $H_4 = 11.180$, p = 0.025, Dunn's test p=0.021) (Figure 3.7). However, no differences in chases or waiting were observed across reefs (Chases: Kruskal-Wallis $H_4 = 6.861$, p = 0.143; Waiting: Kruskal-Wallis $H_4 = 3.157$ p = 0.532).

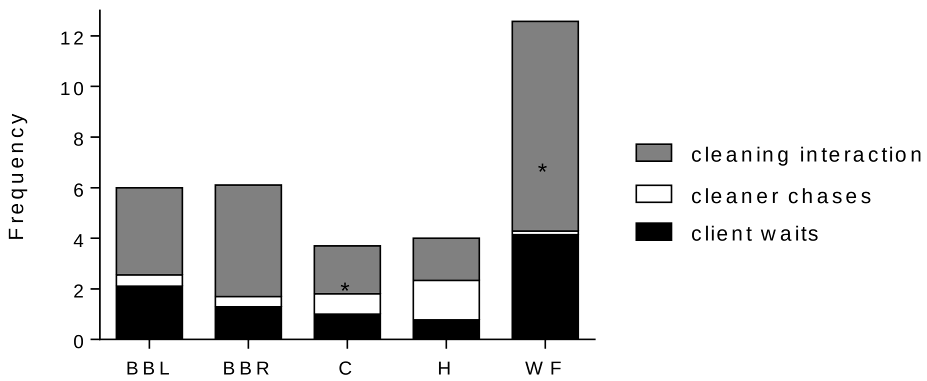

Figure 3.7 – Mean frequency of cleaning interactions, cleaner chases and client waits observed in cleaning stations of single gobies in the five sampled reefs. Significant differences marked with: * Dunn's test p<0.05
Sample size for all variables: n_{WF}=7, n_{BBL}=n_H=9, n_{BBR}=n_C=10.[2]

The average length of single cleaning gobies' clients was in fact different across some reefs (one-way ANOVA, $F_4 = 3.938$, p = 0.009) (Figure 3.8a), however only Blue Bay Left and Habitat were

[1] The species responsible for these differences can be seen in the form of a Table in Appendix I, p 43.
[2] Box-plots of clean, wait and chase frequencies in single CS in Appendix II, p 44.

significantly different (Tukey test, p = 0.006), with Habitat (Mean ± SD = 17.32 ± 8.38) having on average bigger clients than Blue Bay Left (Mean ± SD = 6.88 ± 3.13).

Single gobies cleaning interactions had the same duration between reefs (one-way ANOVA, $F_4 = 1.863$ $p = 0.148$) (Figure 3.8b).

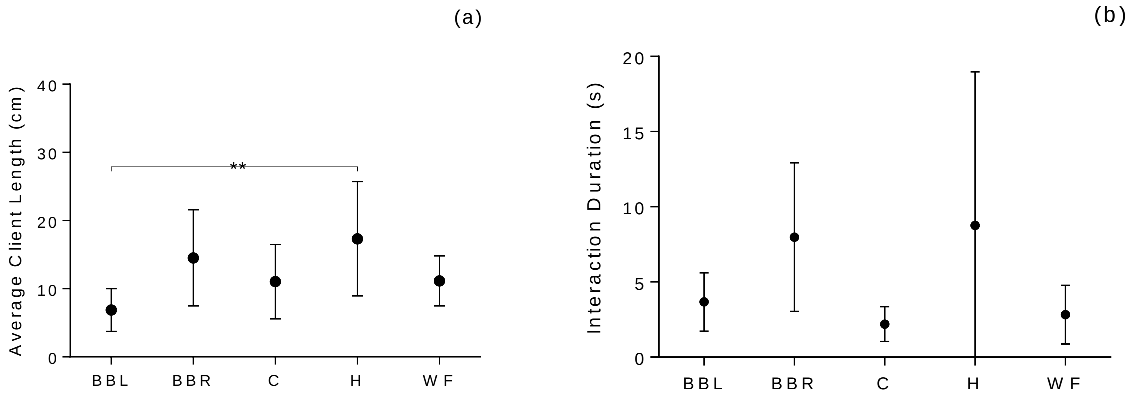

Figure 3.8 – Mean (± SD) average client length (a) and cleaner-client interaction duration (b) at single goby cleaning stations in the five sampled reefs. Significant differences are marked with: ** Tukey test, p<0.01. Sample size for client size and interaction duration respectively: n_{WF}=7, n_{BBL}=n_{H}=9, n_{BBR}=n_{C}=10; n_{C}=4, n_{BBL}=n_{H}= n_{WF}=6, n_{BBR}=8.

No significant differences were found across reefs for client likelihood of posing success (Figure 3.9a), (Kruskal-Wallis H_4 = 2,927; p = 0,570) the proportion of cleaning interactions initiated by the cleaner (Figure 3.9c) (Kruskal-Wallis, H_4 = 6,535; p = 0.163), likelihood of chase by cleaner success (Figure 3.9b) (Kruskal-Wallis, H_4 = 1,808 p = 0.771) and client jolts (Figure 3.9d) (one-way ANOVA, $F_4 = 0.685$, p = 0.608). For both likelihood of chase or posing success, the values range from 0 to 2, in this case 0 means that none of the behaviors were recorded, not allowing the calculation of the likelihood. The value 1 is referring to when all poses/chases were rejected, therefore not leading to a cleaning interaction and the value 2 is when all poses/chases lead to a cleaning interaction.

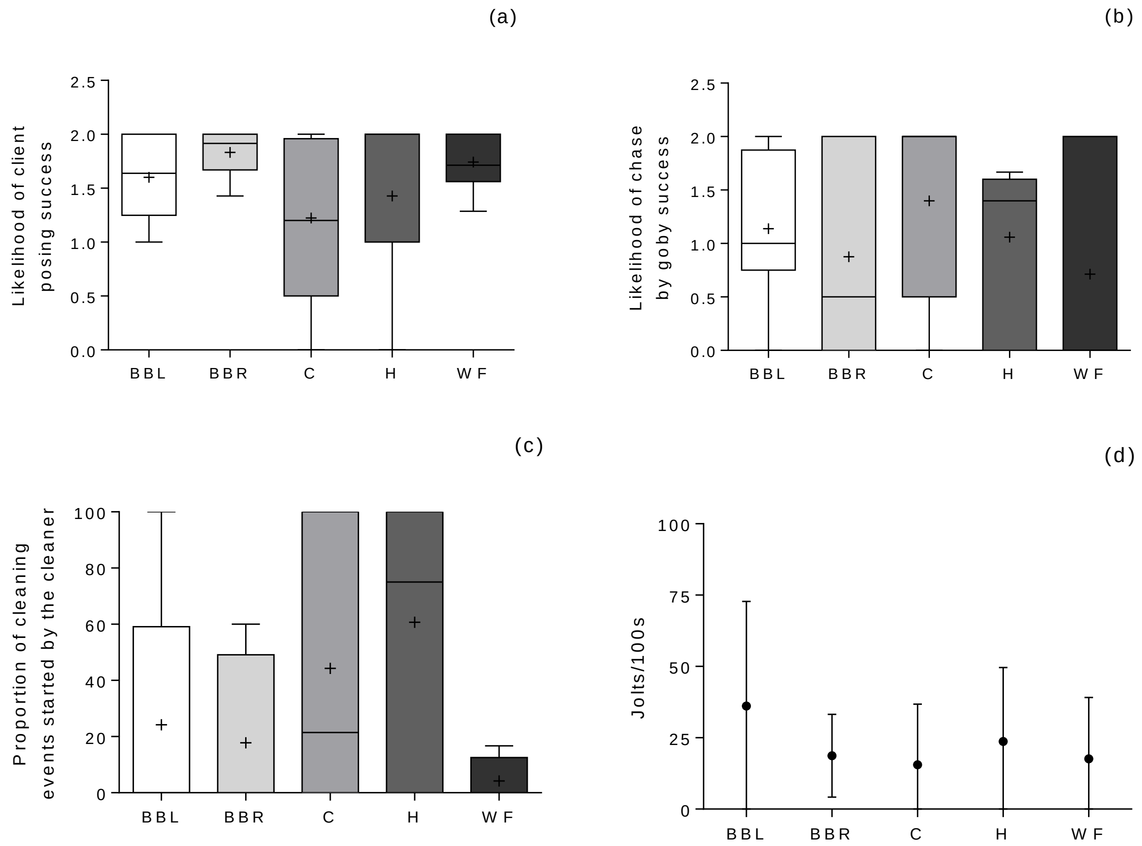

Figure 3.9 - Likelihood of (a) client posing success, (b) chase by a goby success and proportion of cleaning events that started by cleaner initiative (c) at single cleaning stations. Box-plots represent median, minimum, maximum, 1st and 3rd quartiles. Mean is marked with "+".
(d) Mean (±SD) jolts per 100s performed by clients at single cleaning goby stations in the five sampled reefs. Sample size for all variables: n_{WF}=7, n_{BBL}=n_{H}=9, n_{BBR}=n_{C}=10.

From the variables described above (jolts/100 s, likelihood of client posing success, likelihood of chase by goby success, proportion of cleaning events initiated by the cleaner and the average interaction duration), only the proportion of cleaning events initiated by the cleaner seemed to be influenced by the reef identity (GLM, distribution = Normal, link function = Identity, $p = 0.044$). Yet the likelihood of chase by goby success and the proportion of cleaner initiated interactions seemed to be influenced by the average length of the clients positively (GLM, distribution = Normal, link function = Identity, $p = 0.006$; GLM, distribution = Normal, link function = Identity, $p = 0.019$, respectively).

3.2.2. Paired cleaning gobies

No differences were found across reefs regarding cleaning or chase frequency (interaction frequency: Kruskal-Wallis, $H_4 = 6.937$; $p = 0.139$; chase frequency: Kruskal-Wallis, $H_4 = 2.117$, $p = 0.714$) (Figure 3.**10**). Significant differences were solely found regarding the waiting frequency (waiting: Kruskal-Wallis $H_4 = 18.243$, $p = 0.001$). Specifically, these differences were observed between Water Factory and Habitat and Water Factory and Blue Bay Right (Dunn's test, $p = 0.005$ and $p = 0.023$ respectively) where clients were put to wait more frequently (Figure 3.10).

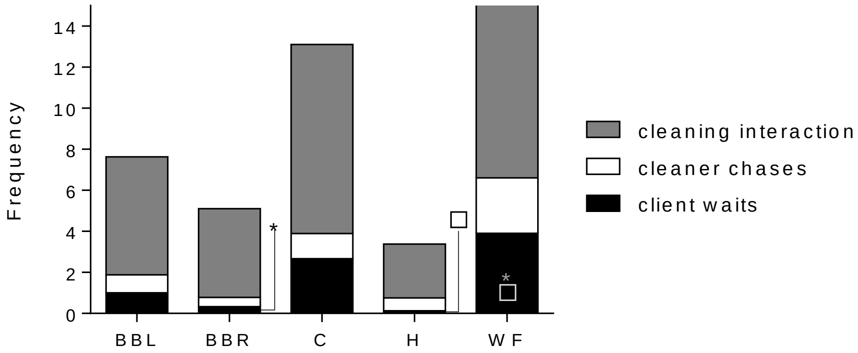

Figure 3.10 - Mean frequency of cleaning interactions, cleaner chases and client waits observed in pair cleaning stations in the five sampled reefs. Significant differences marked with * and □, Dunn's test p<0.05
Sample size for both variables: n_H=6, n_{BBR}=7, n_{BBL}=8, n_H=n_{WF}=9. [3]

The average length of clients (per reef) and the interaction duration were not significantly different across reefs (average client length: Kruskal-Wallis, $H_4 = 6.399$, $p = 0.171$; Interaction duration: one-way ANOVA, $F_4 = 2.158$ $p = 0.098$) (Figure 3.11).

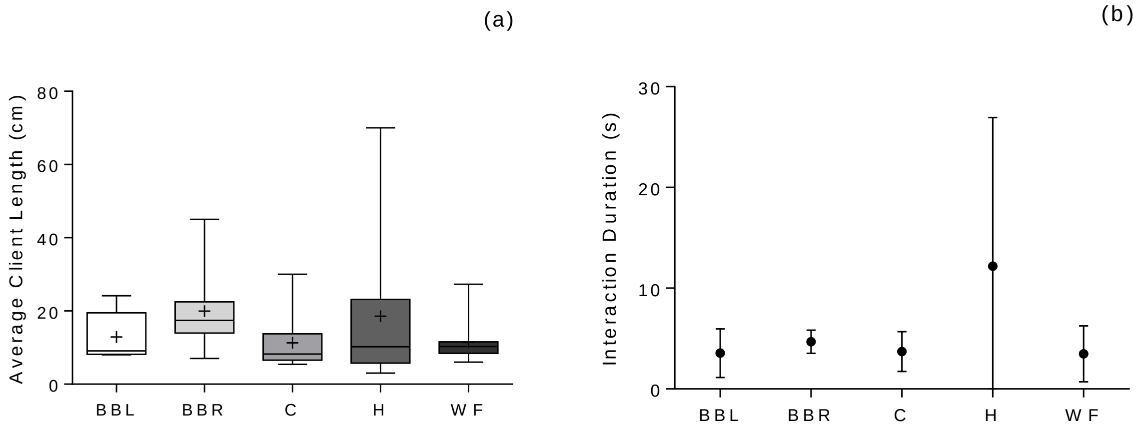

Figure 3.11 – (a) Average length of clients visiting paired cleaning stations in the five sampled reefs. Box-plots represent median, minimum, maximum, 1st and 3rd quartiles. Mean is marked with "+".
(b) Mean (±SD) duration of cleaning interactions in paired gobies cleaning stations.
Sample size for both variables: n_H=6, n_{BBR}=7, n_{BBL}=8, n_H=n_{WF}=9.

Moreover, no differences were found regarding likelihood of chase by goby success, likelihood of client posing success, in the proportion of cleaning interactions initiated by the cleaner and jolts per 100 s (likelihood of chase by goby success: Kruskal-Wallis, $H_4 = 2.795$ p=0.93; likelihood of client posing success: Kruskal-Wallis, $H_4 = 6.197$, $p = 0.185$; proportion of cleaner initiated interactions: Kruskal-Wallis, $H_4 = 3.452$ $p = 0.474$; jolts/100 s: one-way ANOVA, $F_4 = 1.568$, $p = 0.208$)(Figure 3.12). For

[3] Box-plots of clean, wait and chase frequencies in paired CS in Appendix III, p 45.

both likelihood of chase or posing success, the values range from 0 to 2, in this case 0 means that none of the behaviors were recorded, not allowing the calculation of the likelihood. The value 1 is referring to when all poses/chases were rejected, therefore not leading to a cleaning interaction and the value 2 is when all poses/chases lead to a cleaning interaction.

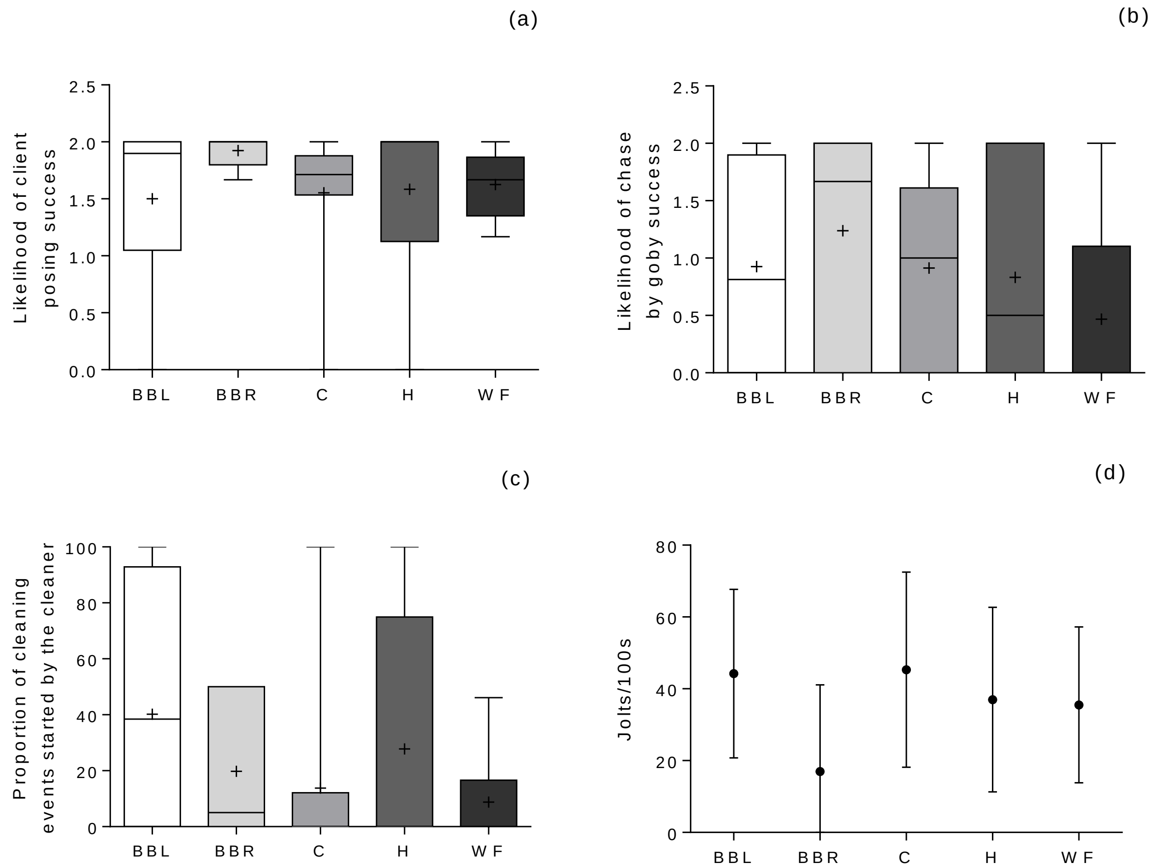

Figure 3.12 – Likelihood of (a) client posing success, (b) chase by a goby success and proportion of cleaning events that started by cleaner initiative (c) at paired cleaning stations. Box-plots represent median, minimum, maximum, 1st and 3rd quartiles. Mean is marked with "+".
(d) Mean (±SD) Jolts per 100 s performed by clients at paired cleaning goby stations in the five sampled reefs. Sample size for all variables: n_H=6, n_{BBR}=7,n_{BBL}=8, n_C=n_{WF}=9.

None of the variables described above seems to be influenced by reef identity (Jolts/100 s GLM, distribution = Normal, Link function = Identity, p = 0.765; Likelihood of chase success GLM, distribution = Normal, Link function = Identity, p = 0.308; Interaction duration GLM, distribution = Normal, Link function = Identity, p = 0.203). However the interaction duration was being influenced by the average client size (GLM, distribution = Normal, Link function = Identity, p = 0.003). Models could not be run for the likelihood of client posing success and for the proportion of cleaning events started by the cleaner.

3.3. Diet analysis

Caligids intake in single cleaning gobies stomachs did not vary between reefs (Kruskal-Wallis, H_4 = 7.819, p = 0.098) (Figure 3.13a). Gobies also did not cheat differently between reefs (one-way ANOVA, F_4 = 0.601, p = 0.667) (Figure 3.13c).The gnathiids on the other hand had significant differences across reefs (one-way ANOVA, F_4 = 9.385, p < 0.001) (Figure 3.13b). These differences were observed between Habitat, that had a higher intake of gnathiids, all the other reef (Tukey test, $p < 0.05$).

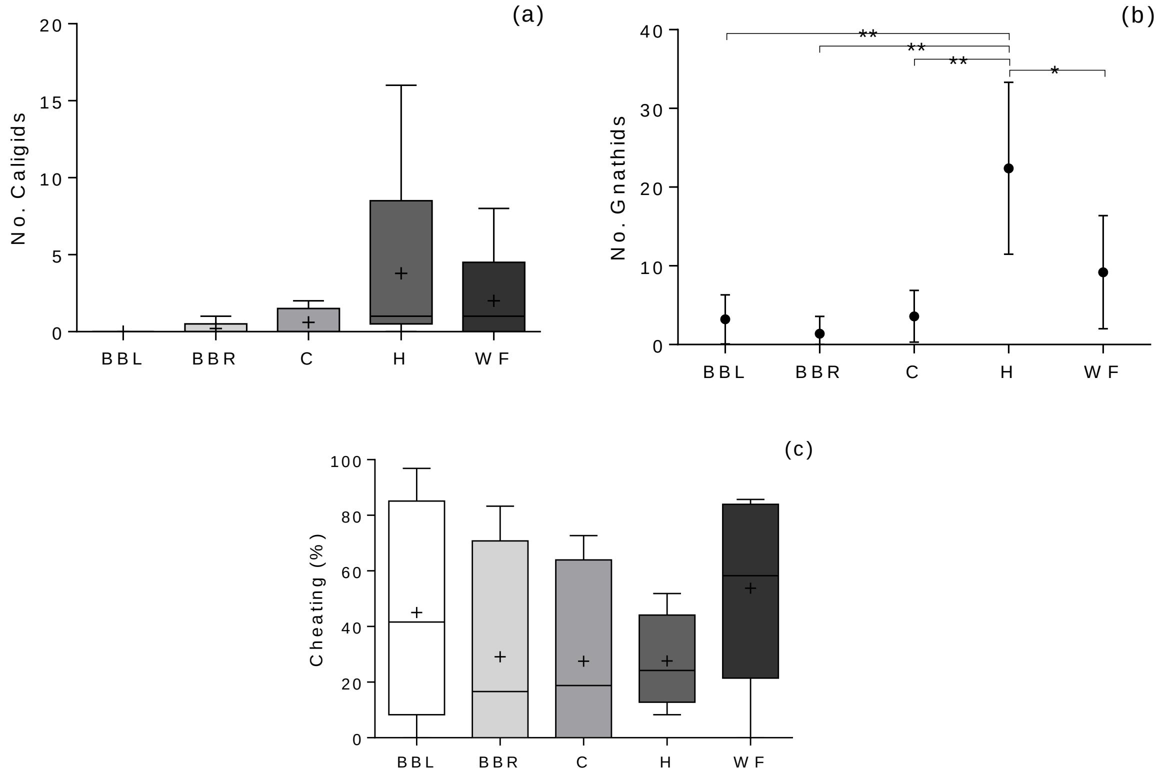

Figure 3.13 – (a) Number of caligids in single cleaning gobies stomachs and percentage of cheating (c) across five different reefs. Box-plots represent median, minimum, maximum, 1st and 3rd quartiles. Mean is marked with "+". (b) Mean (±SD) frequency of gnathiids in single cleaning gobies. Significant differences are marked with: * Tukey test, $p<0.05$, ** Tukey test, $p<0.01$. [4]
Sample size for all variables: $n_{BBL}= n_{BBR}= n_{C}=n_{H} = n_{WF}=5$.

The PERMANOVA results showed that the single cleaning gobies stomach content is being significantly influenced by the reef identity (Pseudo-F = 2,660, p = 0.002) (Table 3.2). The pairwise tests revealed significant differences between Habitat and Blue Bay Left, Blue Bay Right and Carmabi ($p < 0.05$) and between Water Factory and Blue Bay Right and Carmabi ($p < 0.05$) (Table 3.3).

Table 3.2 - Results of the PERMANOVA analysis conducted to compare the stomach content of single cleaning gobies between different reefs. Significant p-values ($p < 0.05$) marked in bold type.

Source	dF	SS	MS	Pseudo-F	P (perm)	Unique Terms
Reef	4	18944	4736	2.6605	**0.002**	999
Res	20	35602	1780.1			
Total	24	54545				

[4] Box-plot of the number of scales found in single gobies in Appendix IV, p 46.

Table 3.3 - Results of Pairwise PERMANOVA comparisons between reefs for single cleaning gobies stomach content (p-values). Significant differences (p < 0.05) in bold type.

Reef	BBL	BBR	C	H
BBL				
BBR	0.603			
C	0.857	0.551		
H	**0.012**	**0.011**	**0.01**	
WF	0.155	**0.017**	**0.038**	0.177

Paired cleaning gobies caligids consumption was similar across all the reefs (Kruskal-Wallis, $H_4 = 4.306$, $p = 0.366$) (Figure 3.14a), and again, reefs had a similar percentage of cheating (Kruskal-Wallis $H4 = 6.678$, $p = 0.154$) (Figure 3.14c). On the other hand, differences across reefs were found for gnathids intake (Kruskal-Wallis, $H_4 = 12.494$, $p = 0.014$;) (Figure 3.14 b). For gnathiids differences were found between Blue Bay Left and Water Factory (Dunn's test, $p = 0.008$), in which the latter had a higher intake than the first.

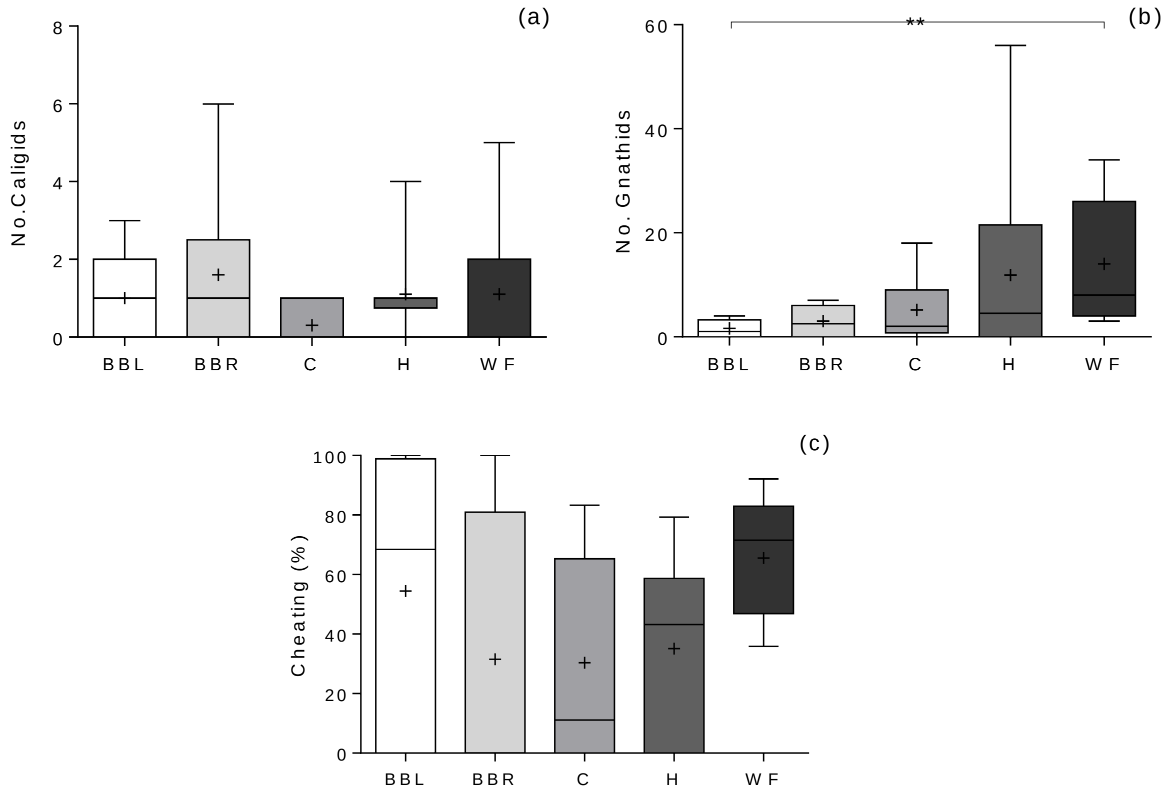

Figure 3.14 –Number of caligids (a) and gnathiids (b)) in paired cleaning gobies stomachs and percentage of cheating (c) across five different reefs. Box-plots represent median, minimum, maximum, 1st and 3rd quartiles. Mean is marked with "+". Significant differences are marked with: ** Dunn's test, p<0.01.[5]
Sample size for all variables: $n_{BBL} = n_{BBR} = n_C = n_H = n_{WF} = 10$.

[5] Box-plot of the number of scales found in paired gobies in Appendix V, p 46.

For paired cleaning gobies stomach content PERMANOVA also demonstrated that these were influenced by the reef identity (Pseudo-F = 2.481, $p = 0.008$) (Table 3.4) and significant differences were found between all reefs and Water Factory ($p < 0.05$) and a lack of differences between all the other reefs (all pairwise tests: $p > 0.05$) (Table 3.5).

Table 3.4 - Results of the PERMANOVA analysis conducted to compare the stomach content of paired cleaning gobies between different reefs. Significant p-values ($p < 0.05$) marked in bold type.

Source	dF	SS	MS	Pseudo-F	P (perm)	Unique Terms
Reef	4	20806	5201.5	2.4815	**0.008**	998
Res	45	94324	2096.1			
Total	49	1.1513e5				

Table 3.5 - Results of Pairwise PERMANOVA comparisons between reefs for paired cleaning gobies stomach content (p-values). Significant differences ($p < 0.05$) in bold type.

Reef	BBL	BBR	C	H
BBL				
BBR	0.342			
C	0.233	0.843		
H	0.603	0.603	0.644	
WF	**0.018**	**0.001**	**0.002**	**0.005**

3.4. Cleaning gobies cortisol levels

Single cleaning gobies cortisol levels are significantly higher in Carmabi than in all the other observed reefs (one-way ANOVA, $F_4 = 6.615$, $p = 0.001$; Tukey test, $p < 0.001$ for all reefs and Carmabi) (Figure 3.15). These differences were being significantly influenced by the reef where the single gobies lived and the number of client species in their cleaning stations (Table 3.6).

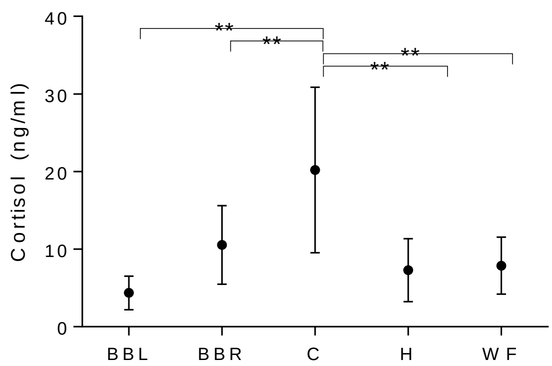

Figure 3.15 –Mean (± SD) cortisol levels in single cleaning gobies across the five sampled reefs. Significant differences marked with: ** Tukey test, $p < 0.01$. Sample size: $n_C=5$, $n_{BBL}=6$, $n_H=n_{WF}=7$, $n_{BBR}=8$.

Table 3.6 – General linear model test effects for cortisol in single gobies as a dependent variable. Significant values are marked with bold type ($p < 0.05$). Model AIC = 215.03.

Variables		Wald Chi-Square	dF	Sig
Reef		27.626	4	**0.000**
	BBL		1	0.463
	BBR		1	0.344
	C		1	**0.000**
	H		1	0.665
Jolts/100s		0.060	1	0.806
Average client size		1.263	1	0.261
Likelihood of chase success		0.455	1	0.500
No. of client species in the cleaning station		4.102	1	**0.043**

Paired cleaning gobies cortisol levels did not vary across reefs (individual A: one-way ANOVA, $F_4 = 0.942$, $p = 0.452$; individual B: one-way ANOVA, $F_4 = 1.868$, $p = 0.139$) (Figure 3.16), Habitat appears as the reef with the highest cortisol levels (individual A, Mean ± SD = 16.74 ± 6.95; individual B, Mean ± SD = 17.99 ± 9.54) and Water Factory the lowest for individual A (Mean ± SD = 9.03 ± 4.07) and Blue Bay Left for individual B (Mean ± SD = 7.98 ± 4.62).

No differences were found between individual A and B inhabiting the same reef (BBL: Mann-Whitney, $U_{16} = 27$, $p = 0.600$; BBR: T-test $T_{12} = -0.046$, $p = 0.964$; C: T-test $T_{16} = 2.113$, $p = 0.051$; H: T-test $T_{10} = -0.259$, $p = 0.801$; WF: Mann-Whitney, $U_{18} = 31$, $p = 0.402$).

Paired gobies cortisol levels were only influenced by the likelihood of chase success, even though Blue Bay Right, Carmabi and Habitat presented p-values below 0.05 it was not enough to prove the reef an influencing factor (Table 3.7).

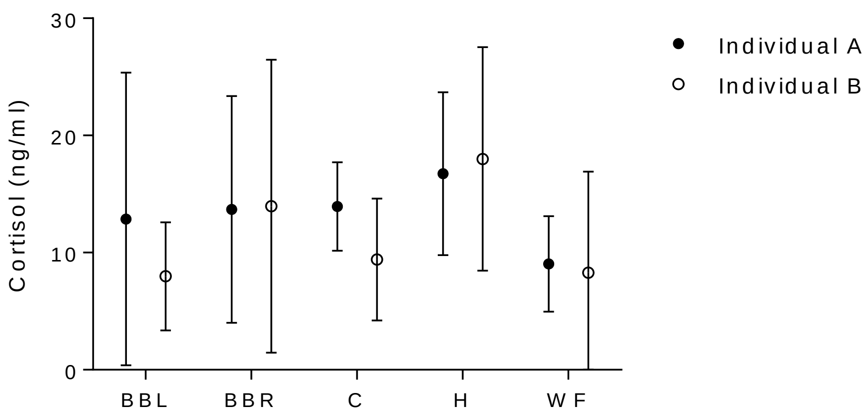

Figure 3.16 – Mean (± SD) cortisol levels in paired cleaning gobies across the five sampled reefs. Sample size for both individuals: n_H=6, n_{BBR}=7, n_{BBL}=8, n_H=n_{WF}=9.

Table 3.7 - General linear model test effects for cortisol in individual A of paired gobies as a dependent variable. Significant values are marked with bold type ($p < 0.05$). Model AIC = 279.960.

Variables		Wald Chi- Square	dF	Sig
Reef		7.310	4	0.120
	BBL	3.082	1	0.079
	BBR	3.948	1	0.047
	C	3.925	1	0.048
	H	5.139	1	0.023
Jolts/100s		0.390	1	0.532
Average client size		0.740	1	0.390
Likelihood of chase success		4.696	1	**0.030**
No. of client species in the cleaning station		1.300	1	0.254

3.5. Inside the reef: a comparison between single and paired cleaning gobies

Tests were run for all behavioral, dietary and physiological variables, comparing single and paired gobies living in the same reef. However, almost no differences were found. In the 80 tests performed, only a few proved to be statistical significant. In Water Factory and Blue Bay Right no differences were found between pairs and singles. For Blue Bay Left, paired gobies had on average larger clients than single gobies (Mann-Whitney, $U_{17} = 60.00$, $p = 0.021$), and paired gobies were more stressed than single gobies (Mann-Whitney, $U_{22} = 78.00$, $p = 0.027$). In Carmabi, paired had more cleaning interactions than single gobies (Mann-Whitney, $U_{19} = 81.00$, $p = 0.002$), clients jolted more at paired cleaning stations (Mann-Whitney, $U_{14} = 38.00$, $p = 0.042$) and single gobies were more stressed than the individual B of the pair (T-test, $T_{12} = 2.585$, $p = 0.024$). Finally, in Habitat, singles were less stressed than paired gobies. (T-test, $T_{16} = -3.638$, $p = 0.002$).

3.6. A brief summary

Below are two tables summarizing all the results obtained. Reefs with significant differences are indicated with a mathematical symbol. When those symbols are different, there were differences between reefs. The signal '+' means that reef(s) had a higher value than the other; the signal '-' indicates that reef(s) had a lower value than the others (*e.g.* % Coral Live Coral: WF is different to all the other reefs, having more percentage of live coral cover than all the other reefs; and C is different than BBL and H, having a lower cover than them). No symbols no differences were found.

For stomach content the test used does not provide the relationship between the reefs, only if they are different or not, so in order to mark significant differences an asterisk is used (the reef with an asterisk '*' and background color is significantly different to the other reefs that are also marked with an asterisk).

Table 3.8 – Summary of reef conditions results.

Studied variable	BBL	BBR	C	H	WF
% Cover Live Coral	-	-	-	-	+
	+		-	+	
% Cover Dead Coral	+		-	+	+
% Cover DC w/ Algae			+		-
% Cover Sand					
% Cover Other					
No. Coral Species	-	-	-	-	+
No. Single Cleaning Station		+		-	
	+	+	-		+
No. Pair Cleaning Station	+			-	+
	+	+	-		+
No. of Cleaning Stations		+		-	+
	+	+	-		+
No. *E. evelynae*		+		-	+
	+	+	-		+
Fish density		+	-	+	
No. Fish Species		+			-
	+	+	-		

Table 3.9 – Summary of behavioral, dietary and stress results.

Studied variable	Org.	BBL	BBR	C	H	WF	Influencing variables
Cleaning Interaction Frequency	Single			-		+	
	Pair						
Chases Frequency	Single						
	Pair						
Waits Frequency	Single						
	Pair		-		-	+	
Average Client Lengh	Single	-			+		
	Pair						
Interaction Duration	Single						
	Pair						Average client size
Likelihood of client posing sucess	Single						
	Pair						
Likelihood of chase by goby sucess	Single						Average client size
	Pair						
Proportion of cleaning events started by the cleaner	Single						Reef and Average client size
	Pair						
Jolts/100s	Single						
	Pair						
Client Species Frequency at cleaning station	Single						
	Pair	*	*	*			
		*	*	*	*	*	
Caligids Frequency	Single						
	Pair						
Gnathids Frequency	Single	-	-	-	+	-	
	Pair	-				+	
Scales Frequency	Single						
	Pair	+	-			+	
Cheating (%)	Single						
	Pair						
Stomach content (gnathids, caligids, scales)	Single	*	*	*	*		Reef
			*	*		*	
	Pair	*	*	*	*	*	Reef
Cortisol	Single	-	-	+	-	-	Reef and no. of client species
	Pair- IndA						Likelihood of chase by goby sucess
	Pair- IndB						
	IndA vs B						

4. DISCUSSION

Taking into consideration the changes presently faced by coral reefs and the importance that cleaning gobies have in these habitats, this study aimed to understand if the ecology of gobies living in reefs with different health conditions reflected those differences. To assess this impact five questions were asked: 1) What is the health status of the studies reefs?; 2) Do cleaning gobies have different food consumption between reefs?; 3) Do gobies behave differently between reefs?; 4) Are gobies from different reefs subjected to different stress levels?; 5) Are these food, behavioral and stress differences due to health status of the reef they inhabit.

4.1. The health status of the studied reefs

When compared with other islands in the Caribbean, Curaçao has one of the best preserved reef systems, yet they too are faced with degradation (Vermeij 2012). This makes them an ideal place (*i.e.* natural laboratory) to study the impact that different degrees of reef degradation may have on the main cleaner fish species, *E. evelynae*.

Concerning coral related data, it is clear that Water Factory is the healthiest reef among the five reefs sampled. It has the higher live coral percentage cover and diversity when compared with the other reefs. Contrarily, Carmabi is the most degraded reef, with lower diversity and cover of live coral. In addition, Carmabi had a large amount of dead coral cover with algae, with only a small portion of it not being covered, suggesting the reef is already in an advanced degradation stage. The remaining reefs did not stand out from one another.

Regarding to the fish communities, Water Factory and Carmabi were the least diverse and dense reefs. The fact that Water Factory had such low values was unexpected since there is usually an association between high coral cover and high fish density and diversity levels (Bell and Galzin 1984). This might be explained by reef location. Water Factory is situated right beside a fishing port, thus possibly being fished upon. It is also the closest reef to Willemstad, where the lowest fish abundance occurs (Vermeij 2012).

On the other hand, Carmabi results were not surprising, as contrarily to Water Factory, it has a low coral cover. It is also located next to a freshwater canal, which creates turbidity in the reef, that according to Bejarano and Appeldoorn (2013) can negatively impact fish communities. No differences were found between Habitat and Blue Bay Right and Left, which had high fish diversity and density.

Taking both coral and fish communities into consideration, one should be able to reach the reef health status. Carmabi provides the clearest signal as the reef in poor health conditions, with both low coral and fish density and diversity. Water Factory, even with its smaller fish community, may be considered the reef in better health conditions overall. These findings are in line with Vermeij (2012) assessment where Water Factory is described as one of the remaining reefs in the island that it is still forming and/or renewing reef structure. Also in this report, Carmabi was described as facing severe degradation in the past three decades, Habitat as a reef where decline is currently occurring at a fast rate and Water Factory as a reef with a moderate decline. This report did not present data for Blue Bay (Left or Right) but the reefs in that area were facing degradation as well.

Regarding *E. evelynae*, Carmabi and Habitat had the lowest densities; while in Habitat this can lead to an increase in competition between clients to access cleaning stations (Soares et al. 2008b), in the case of Water Factory the opposite may occur, since it has a high density of gobies but a low density of clients.

Coral Health Index (CHI) and Reef Health Index (RHI) are two relevant tools to evaluate the health of any given reef in the form of an index. However, it takes into consideration not only the density and diversity of fish communities but also their mass (Carruthers et al. 2011; Díaz-Pérez et al. 2016). This parameter was not recorded in the sampled data, and thus the inability to obtain this index. In addition to this, CHI can also have in consideration the microbial community, which was not measured in this study.

Hence, for the purpose of this study, Water Factory was considered a healthy reef, Habitat, Blue Bay Left and Blue Bay Right fair reefs and Carmabi a degraded reef.

Although some conclusions about the health of the sampled reefs can be made, it would be ideal that in the future these variables could also be measured. Especially in a study dealing with cleaning gobies where the parasite emergence rate is so important. This would have allowed a better distinction between reefs, the establishment of a gradient of reef health and better comparison with other studies (Carruthers et al. 2011).

4.2. Do cleaning gobies have different food consumption between reefs?

In this study only gnathiids showed variation between reefs in terms of stomach content in both single and paired goby cleaning stations. For single gobies, Habitat was the reef with higher gnathiids intake, while for paired gobies Water Factory had the higher values, but was only different from Blue Bay Left. It was expected that more differences would occur since it was expected that different reef conditions would result in different infestation levels, which would be reflected by differences in feeding content. In fact, a recent study by Santos (2016), found differences in clients' parasite loads between Carmabi and Water Factory. However, they were due to the Monogenea family, which can be difficult to find in stomach contents since they have a soft and easy to digest body (Becker and Grutter 2004).

It could be expected that gobies that had eaten a lower quantity of parasites may have eaten more scales. However, this did not happen. No differences for scales intake were observed for single gobies. In pairs Water Factory and Blue Bay Left had more scales than Blue Bay Right. Since gobies prefer parasites as a food item, only switching to scales when the parasite loads are depleted (Soares et al. 2010; Arnal and Côté 2000; Grutter 2002; Soares et al. 2010), the higher intake of scales by gobies in Water Factory supports the idea that Water Factory clients were not highly parasitized. However, this could be a cycle, where fish are not highly parasitized because they visit cleaning stations regularly and do not let the parasite loads increase to less than ideal levels.

The percentage of cheating did not vary between reefs for either single or pair gobies, which can be due to the higher intake of scales being accompanied with a higher parasite intake, and thus not translating into a higher cheating rate. This can be related to gobies not wanting scales as a food item, only using them as a sign that clients should end the interaction as proposed by Soares et al. (2008), or with a putative lower statistical power of non-parametric analysis.

So, does a high number of parasites in the gobies' stomachs means that the clients are highly parasitized? The following two hypotheses are suggested concerning stomachs with high amount of parasites: 1) clients are indeed highly parasitized and thus even with low cleaning interactions gobies can access such high quantities; or 2) clients are not highly parasitized but the high number of interactions allows gobies to get access to high parasite quantities. In order to completely answer this, the stomach content should be associated with the goby behavior, to know if, in fact, the gobies had a high or low number of cleaning interactions or, if a low number of interactions was associated with a high parasite ingestion (the clients had a high parasite load). Additionally, parasite emergence rates and clients' parasite loads should be sampled, to better understand the conditions occurring in that reef. Unfortunately, it was not logistically possible to sample those two variables, or to have the same goby providing both behavioral and dietary information.

Although few differences were identified between reefs in terms of different food items, when taking all information into consideration, single gobies in Habitat and Water Factory show different diet preferences from almost all reefs, and Water Factory differs from all reefs in paired gobies.

Reef identity appears as an influencing factor for the existing variations. These differences in results (single food item vs. all stomach content) might be related to the statistical tests used, and the fact that the variations in each food item were not enough for the test to identify statistical differences individually, but, when all combined those variations are amplified and thus the capacity to differentiate the reefs increases as well.

As the sample size for the stomach analysis was small, it might not be representative of the population. Therefore, all these results must be considered only as an indicative of what might be happening in Curaçao reefs and as an incentive to do a more intensive sampling effort in this matter.

4.3. Do gobies behave differently between reefs?

Gobies' behavior can be broken down into numerous components, although the number of cleaning interactions, frequency of waiting events and chases may be considered the center of all these behavior variables.

Single gobies in Water Factory had a higher number of cleaning interactions than those in Carmabi. However, for paired gobies, such difference did not occur between reefs. This is due mostly to the increase of paired cleaning interactions in Carmabi. So much so, that this difference (in cleaning interaction frequency) between pairs and singles in Carmabi proved to be significant, being one of the few differences happening between pairs and singles of the same reef. This can be an indication that in degraded reefs it could be more advantageous for gobies to have a partner. Clients in degraded reefs might prefer cleaning stations where paired gobies clean, since it has been described that cleaning in pairs increases honesty (Soares et al. 2009). This lowers the risk of clients being negatively affected by the interaction as that could have a larger impact in the clients' health, which may already be less than ideal as they inhabit a degraded reef (Fausch et al. 1990).

No differences in cleaning interactions were found between the healthy (Water Factory) and the fair reefs (Blue Bay Left, Blue Bay Right, Habitat). This may be due to reef conditions not being different enough, as Water Factory is also facing some level of degradation (Vermeij 2012). Thus, the fair and healthy reefs conditions differences might not be enough to translate into differences in goby behavior between them, contrarily to Carmabi and Water Factory, as they have the highest overall dissimilarity between reefs.

The fact that the differences observed for the number of cleaning interactions did not match the ones of the stomach contents can be related to the causes referred in the previous section: the individuals observed and caught were not the same, and a low sample size. Additionally, it can also be related with the gobies time of capture. Gnathiids have a daily emergence pattern (Chambers and Sikkel 2002; Sikkel et al. 2006, 2009), affecting goby activity, and while behavior observations were done throughout the day, gobies sampled for stomach analysis were caught all at once, but at different hours between reefs. It is possible then that stomach contents from higher and lower periods of activity were collected, introducing bias in the comparisons between reefs.

Nevertheless, in Habitat reef single gobies ate a high number of parasites and had few cleaning interactions. While Water Factory gobies had a high rate of cleaning interactions and a low intake of parasites, supporting the hypothesis suggested, in which high parasite content in gobies' stomachs could be associated with a low number of cleaning interactions, due to the clients' higher parasite loads.

The underlying motivations for cleaners and clients may be best represented by chase and wait frequency, likelihood of chase and wait success, and the proportion of cleaning events started by the cleaner. For cleaners, chase frequency is related to their eagerness to start an interaction, and the likelihood of success for client posing is the willingness of the cleaner to clean. For the client, the contrary occurs. The willingness to wait represents the desire to be cleaned, and the likelihood of chase success is related to the clients' willingness of being cleaned. The proportion of cleaning interactions started by the cleaner although representing in its majority the eagerness to clean by the gobies, it is also influenced by the clients' motivations.

Once more, it was expected that the different conditions in the reefs would provide differences in the motivations of both sides as Arnal et al. (2001) reported that the number of gnathiid ectoparasites proved to be a good predictor of visits to cleaning stations, with clients with higher loads visiting the cleaning stations more often. However, Arnal et al. (2001) also reported that gobies did not prefer clients with high parasite loads. Proposing that perhaps gobies would prefer clients with parasites that are more easily detected and removed. Being able to maintain a positive interaction even in reefs with low parasite loads. This might be why no differences in motivation driven behaviors were found in both pairs and singles, aside from the higher client wait frequency in Water Factory for paired gobies.

The increase in the likelihood of being cleaned by firstly performing a pose might variate with client species, as so would the benefit of posing (Arnal et al. 2001; Côté et al. 1998). This can also be a reason for the lack of differences for these behaviors in the studied reefs as they have different fish communities. Nevertheless, it can also be that reef condition does not have an impact on these behaviors, at least for the quality levels represented in this study.

Clients' parasitic load and their size can influence the duration of a cleaning interaction and the jolt rates (Arnal et al. 2001; Soares et al. 2008a). Though a bigger client could lead to a longer interaction, since more time would be needed to inspect the whole body surface, an interaction with a client with high parasite loads could also be shorter since the goby would become satisfied faster (Grutter 1995). The fact that no differences were found between reefs in the interaction duration can therefore be related to this. Since no differences in cheating were found, the fact that no differences are registered in jolts per 100s was expected.

The client's average length was recorded not only to verify the existence of differences between reefs but also to possibly justify any behavioral results. However, the only difference observed was between Habitat and Blue Bay Left in cleaning stations with single gobies. So, the graphically higher duration of interactions in Habitat might relate to their bigger clients, and the high standard deviation is due to a large client being cleaned for a long time.

Client size positively influences the interaction duration in paired gobies and the proportion of interactions started by the cleaner and the cleaner chase success in single gobies. This role might be due to cleaners being able to estimate from a distance the clients' surface area, but not being able to do so for the parasite loads, which need a brief body scan to get that information (Grutter 1995). Hence, since larger clients have a larger body surface the total number of parasites might be superior when compared with smaller clients, being more advantageous for the gobies to clean.

The GLM found no influence for reef identity for most behavioral variables, except for the percentage of cleaning interactions started by the single gobies. This strengthens the hypothesis that in fact the lack of differences in behaviors between reefs is a real result and gobies behavior is not affected by the reef conditions. Nevertheless, since so many variables are involved, and some were not accounted for in this study, perhaps having an effect in the results, more sampling effort should be made with the aim to avoid these gaps. For instance, a method to exclude the possible effect of gnathiid emergence in goby activity is to simply sample in a shorter period of time, only comprehending a period of high activity rate, which is referred by Sikkel et al. (2004) to be between 05:45 am and 06:30 am.

4.4. Are gobies from different reefs subjected to different stress levels?

Single gobies from the most degraded reef, Carmabi, had higher body cortisol levels (*i.e.* were more stressed) than the gobies from the healthy and fair reefs. This is one of the first evidences that gobies can be negatively impacted by the conditions of the reef they live in. The GLM supports this hypothesis, as it identified the reef being a significant factor for the variations observed in cortisol levels. However, it was not possible to test if and which reef condition variable had a greater impact in the gobies' stress levels.

These results were expected. However, differences between reefs in fair and healthy condition were also expected, yet were absent. This fact might be due to reef conditions being too similar. Also, the fact that so many factors make up the condition of a reef might lessen the differences between reefs, since the possible combinations of factors with positive or negative influence values are immense. Furthermore, it is not known the threshold beyond which reef conditions start affecting the gobies' stress levels. Thus, it is possible that reefs in fair condition do not promote a stress increase in gobies, since even though they are degraded, they still offer enough conditions for the gobies to thrive.

The number of client species that visited the cleaning stations also seemed to be an impactful variable in the single gobies stress levels, in which more diversity leads to a lower stress level. However, differences in susceptibility to parasitism in each reef group of species may be determining lower stress levels and not the sheer amount of diversity.

Surprisingly, when gobies clean as a pair no differences in cortisol levels were found between reefs. When differences in cortisol levels between singles and pairs of the same reef were found, pairs had a higher cortisol level (Habitat and Blue Bay Left), with the exception in Carmabi, where singles were more stressed.

Very little is known about the baseline stress levels in *E. evelynae*, so these results seem to be the first evidence that show that cortisol basal levels between pairs and singles are different and that their response to reef degradation may also differ. This seems another indication that it might be more advantageous for gobies to have a partner in more degraded reefs, since in Carmabi pairs have a lower level of cortisol than the singles. This might be associated with paired gobies having more cleaning interactions than single ones, and perhaps the access to food also plays a role in gobies stress levels.

In paired gobies, the likelihood of chase by goby success affects the cortisol levels, so if the chance of success is higher gobies have a lower cortisol level. This can be related to a higher success rate translates to a higher willingness of clients to be cleaned and thus more cleaning interactions, leading to more access to food.

Having all this in consideration, in subsequent studies more reefs should be sampled and with a broader spectrum, from very healthy to very degraded. This way it would be possible to better pinpoint when reef conditions start affecting stress levels in gobies. Additionally, it would also be relevant to analyze if there is a more impactful variable of the reefs' condition on gobies stress response.

5. FINAL REMARKS

The human population is increasing anthropogenic pressures all around the globe. Thus, it is important to understand the consequences and severity such pressures have on the environment. With climate change, coral reefs became under severe degradation. This affects not only the species living in the reef but also the human population, since reefs are responsible for various services to us, such as protection against storms, coastal erosion and waterborne diseases, among many others (Moberg and Folke 1999).

Cleaner fish have an active role in the ecosystem not only directly through the cleaning interactions but also in the local diversity of both clients and non-clients (Bshary 2003). Therefore, it is important to understand how reef degradation is affecting these organisms and subsequently the social dynamics in reef communities.

Although the cleaning system is fairly well known and an increasing scientific work has been produced in the last decades (Grutter 1999, 2002, 2008; Côté 2000; Sikkel et al. 2000; Cheney and Côté 2003, 2005, Soares et al. 2008a, 2010; Campos and Sá-Oliveira 2011), there are still a lot of gaps to be understood and researched. As Poulin (1993) stated, a lot of studies create more questions than the ones they answer.

The present study tried to establish the link between reef degradation and change in behavior, stress and diet in cleaning gobies.

No relation was observed between the health conditions of reefs and cleaning gobies behavior. Contrarily, for both diet and stress levels the quality of the reef they inhabit was an important factor. However, the relation was to the reef as a whole, not being possible to identify if a certain characteristic was more impactful than the others. This can be pursued in future projects, as more specific actions on reef ecology should be undertaken.

This study provided the first evidence that cleaning gobies are being negatively affected by reef degradation, becoming more stressed. Not only that, but also that single and paired gobies might have a different stress reaction to reef degradation. Yet, there is still a need for validation in this problem, since it is a very complex subject and there are many variables at play.

The fact that this study compared more than two reefs, sampling not only the two reefs in opposite reef health, but also reefs with some level of degradation, avoids the wrong assumption that cortisol levels in gobies and reef degradation have a linear relationship, in which the more degraded a reef is, the more stressed gobies become. This not only reinforces the complexity of this problem, but also poses a question: how poor does the reef health need to be to start increasing cleaning gobies stress levels? So far, this study indicates that it should be some level between a fair and a degraded reef.

In order to completely answer this, future studies are needed. It is necessary to sample a larger number of reefs, not only increasing the spectrum of degradation, but also increasing the number of reefs per category of degradation. This way, we can assure that these results were not local, and are indeed spread in a much broader scale. In addition, this will allow to better determine from which level of degradation reef health starts affecting gobies stress levels, diet, and if it eventually does affect their cooperative behavior. In the future, studies should also be more inclusive, sampling the clients' ectoparasite loads and sampling the same goby for behavior, stress and diet.

Having all this in consideration, this work sheds new light about this issue, and should be seen as an incentive to pursue more knowledge about this problem.(Idjadi and Edmunds 2006)

6. REFERENCES

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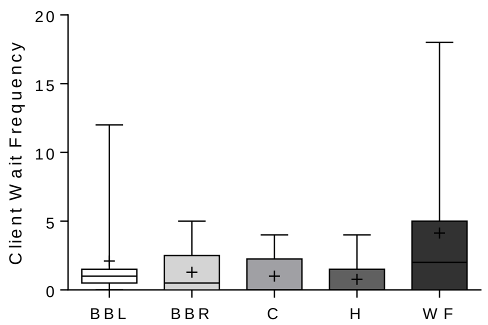
7. APPENDICES

APPENDIX I - SIMPER results showing the responsible fish species for the different fish diversity frequency in paired cleaning stations among the studied reefs. The presented species represent more than 50% of the dissimilarity between reefs.

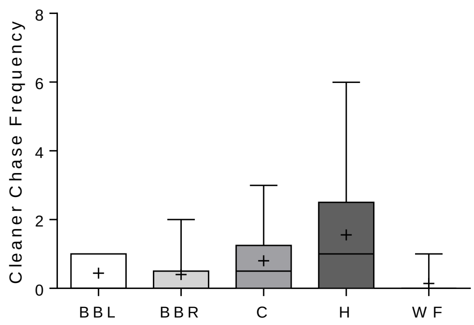
C-BBL	Cumulative % of dissimilarity	C-BBR	Cumulative % of dissimilarity	WF-BBL	Cumulative % of dissimilarity	WF-BBR	Cumulative % of dissimilarity	WF-C	Cumulative % of dissimilarity	WF-H	Cumulative % of dissimilarity
Chromis multilineata	18,45	*Chromis multilineata*	15,71	*Chaetodon capistratus*	19,88	*Cephalopholis fulva*	14,02	*Chromis multilineata*	22,92	*Stegastes partitus*	27,8
Chaetodon capistratus	32,79	*Cephalopholis fulva*	28,51	*Chromis multilineata*	38,44	*Chromis multilineata*	26,22	*Stegastes partitus*	34,76	*Chromis multilineata*	50,57
Chromis cyanea	43,32	*cephalopholis cruentata*	38,44	*Stegastes partitus*	53,15	*cephalopholis cruentata*	38,14	*Chromis cyanea*	46,02		
Scarus taeniopterus	50,95	*Halichoeres garnoti*	48,01			*Halichoeres garnoti*	49,86	*Aulostomus maculatus*	57,26		
		Chromis cyanea	57,04			*Stegastes partitus*	61,49				

APPENDIX II - Frequency of: client waits (a), cleaner chases (b) and cleaning interactions (c) in single cleaning gobies across the five sampled reefs. Box-plots represent median, minimum, maximum, 1st and 3rd quartiles. Mean is marked with “+”. Significant differences for Kruskal-Wallis $H_4 = 11.180$, $p = 0.025$, are marked with: * Dunn’s test $p < 0.05$

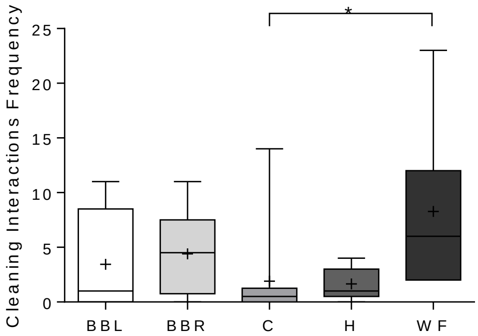
(A)



(B)

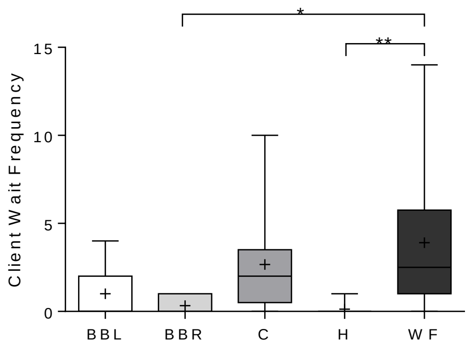


(C)

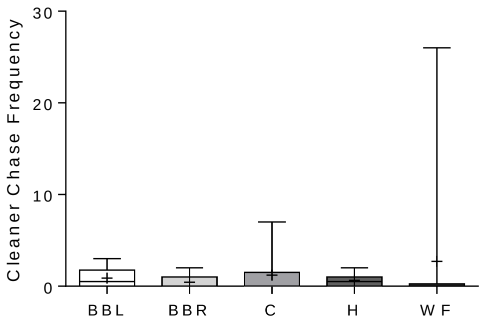


APPENDIX III - Frequency of: client waits (a), cleaner chases (b) and cleaning interactions (c) in paired cleaning gobies across the five sampled reefs. Box-plots represent median, minimum, maximum, 1st and 3rd quartiles. Mean is marked with "+". Significant differences for Kruskal-Wallis $H_4 = 18.243$, $p = 0.001$, are marked with: * Dunn's test $p < 0.05$, ** Dunn's test $p < 0.01$

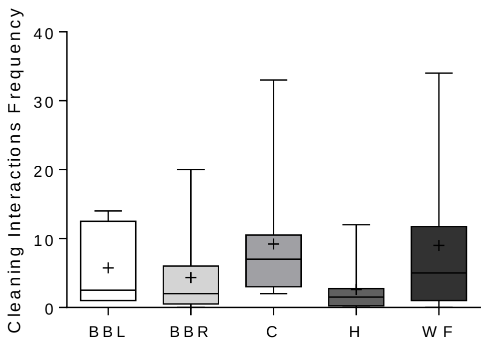
(A)



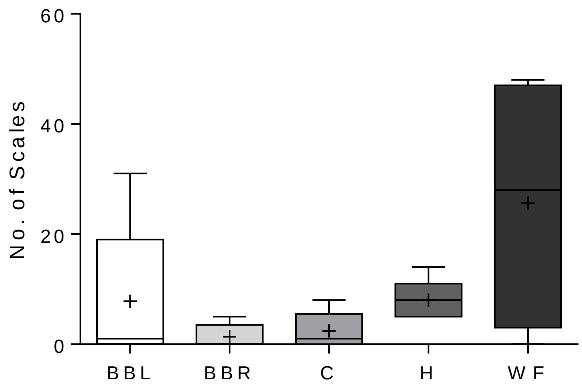
(B)



(C)



APPENDIX IV – Number of scales in single cleaning gobies stomach content across the five sampled reefs. Box-plots represent median, minimum, maximum, 1st and 3rd quartiles. Mean is marked with “+”. No differences were found.



APPENDIX V – Number of scales in paired cleaning gobies stomach content across the five sampled reefs. Box-plots represent median, minimum, maximum, 1st and 3rd quartiles. Mean is marked with “+”. Significant differences are marked with: ** Kruskal-Wallis $H_4 = 17.381$, $p = 0.002$, Dunn’s test, $p < 0.01$